Antagonism of Siderophore Producing Bacteria Against Blood Disease Bacteria

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Abstract. Siderophore is a chemical compound of Fe chelating. This compound is produced by several microorganisms that grow under iron-limiting conditions. Siderophore can facilitate the transfer of Fe from the environment to become available to plants. The siderophore ability to bind Fe as a competitor against other microorganisms, so that in the agricultural system it can be used as a plant disease controller. This study aims to determine the production of siderophore produced by several fluorescent pseudomonad isolates and its antagonistic tests against Blood Disease Bacteria (BDB). Siderophore detection is determined by the absorbance value obtained, and measured using a spectrophotometer at a wavelength of 410 nm. The antagonist test used a completely randomized design with 7 treatments and 3 replications. The treatment is fluorescent pseudomonads isolates PfLAHP2, PfPb1, PfCas3, PfKd7, PfCas, PfPj1, and PfPj2. PfLAHP2 isolates produced the highest siderophore, which was 1.027, and the lowest isolate PfCas was 0.148. The antagonistic test of fluorescent pseudomonad against BDB showed that PfLAHP2 isolate produced the largest inhibitory zone, which was 1.042 cm.

1. Introduction
Banana is a tropical fruit plant that has high economic potential and value, especially for countries in the tropics such as Indonesia. However, the main obstacle limiting banana production is the high rate of disease attacks, including blood diseases caused by Blood Disease Bacteria (BDB). Stunsbury et al., [1] stated that this pathogen is difficult to control because it is soil contagious, and can be spread by flower visitor insects. In addition, BDB attacks banana plants at various growth stages, and infects roots and rhizomes (weevils) through mechanical injury to banana seeds / humps.

The use of biological agents as biological control of plant pathogens recently has been considered a potential control strategy in efforts to control plant diseases. Usually, farmers use synthetic pesticides to control plant diseases. Synthetic pesticides are nonselective, so that the impact is the destruction of pathogenic and non-pathogenic microorganisms, as well as causing microorganisms arising from these pesticides. In addition, synthetic pesticides produce accumulations of hazardous chemical residues, and can cause serious environmental problems. However, indiscriminate and continuous use of synthetic pesticides can endanger human and animal health due to residual poisoning [2].

The most effective control of banana plant diseases is to use resistant cultivars. However, to date no banana cultivars have been found that are resistant to BDB. Therefore we need to find safer and more environmentally friendly control methods. One of the alternative to control BDB that causes banana plant wilting which is environment friendly is to optimize the function of biological agents.
The mechanism that beneficial from using biological agents is by directly utilizing the antagonistic relationship between pathogens and host (hyperparasitism, antibiosis, competition), or indirectly (plant growth booster, induction of endurance) [3]. The utilization of fluorescent pseudomonad biological agents is one alternative for controlling wilt disease bacterial that is still environment friendly. Advinda et al., [4] reported that fluorescent pseudomonad isolates PfPj1, PfPj2, and PfPj3 were able to suppress BDB attacks on Barangan banana seedlings through increased activity of the phenylalanine ammonia-lyase (FAL) enzyme and peroxidase (PO) as plant resistance enzymes.

Fluorescent pseudomonad from the root zone of several types of plants was successfully isolated. 10 fluorescent pseudomonad isolates that had different physiological characteristics from the quality of the fluorescent pigments produced were obtained [5]. According to Advinda [6] fluorescent pseudomonad isolate Mi.1 is the best in controlling BDB in vitro. Furthermore Advinda et al., [7] reported that phytoalexin from the type of cinnamic acid was produced in the roots of banana seedlings that were introduced with fluorescent pseudomonad isolates PfPj1 and BDB inoculation.

One of the compounds produced by antagonistic bacteria is siderophore. So far, there is not many studies have revealed the role of siderophore-producing bacteria in controlling bacterial wilt disease. Siderophore is a chelating agent of iron. This compound is produced by several microorganisms that grow under iron-limiting environmental conditions. Siderophore has a low molecular weight (<1,000Da) and few peptide molecules [6]. According to Patel et al., [9], the ability of iron siderophore to bind is a competitor to other microorganisms, so that in the agricultural system can be used as a control of plant diseases, as well as improving plant growth.

Pseudomonas aeruginosa strain JAS-25 can control several plant pathogenic fungi. Siderophore produced by P. aeruginosa strain JAS-25 can inhibit conidia germination, and degrade mycelium from Fusarium oxysporumf. sp. ciceri, F. udum, and Aspergillus niger [10]. This study aims to determine the production of siderophores produced by several fluorescent pseudomonad isolates and their antagonistic test against Blood Disease Bacteria (BDB).

2. Materials and Methods

This research is a qualitative experimental study including the observation of the ability of bacteria to produce siderophores, and quantitative experiments regarding their antagonistic test against Blood Disease Bacteria (BDB). Fluorescent pseudomonad used are isolates of PfPj1, PfPb1, PfPj2, PfKd7, PfCas, PfCas3, and PfLAHp2.

2.1. Rejuvenation and Propagation of Fluorescent Pseudomonad

Fluorescent pseudomonad isolates PfPj1, PfPb1, PfPj2, PfKd7, PfCas, PfCas3, and PfLAHp2 were rejuvenated on solid King’s B medium, and incubated for 2x24 hours. The inoculum propagation was carried out by taking a pure culture ose in petri, then bred in 25 mL King’s B liquid medium, and is shake for 24 hours.

2.2. The Siderophore Capability Test Media Creation

The siderophore production media consist of 20 g of sucrose, 2 g of L-asparagine, 1 g of K2HPO4, and 0.5 g of MgSO4 dissolved into aquades up to a volume of 1.000 mL. The media sterilization was done using an autoclave at 121° C and 15 psi of pressure for 15 minutes.

2.3. The Siderophore Capability Test Media Creation

The suspension of each fluorescent pseudomonad isolate was taken as much as 1 mL, then transferred to 25 mL of the siderophore test medium, and was shake for 24 hours at room temperature. The resulting suspension was taken as much as 10 mL which then was centrifuged at 3,000 rpm for 10 minutes. The supernatant was taken using micropipette. Detection of siderophore production was carried out by adding 1 mL of FeCl 0.01 M into 3 mL of the supernatant, while the control was used supernatant without the addition of FeCl. Detection of siderophore was measured with a spectrophotometer at 410 nm wavelength [10].
2.4. Fluorescent Pseudomonad Antagonist Test Against BDB
One mL of BDB suspension (density of 108 cells / mL based on 1 McFarland's scale) was inoculated on NA medium in a petri dish. 4 sheets of sterile disk paper were taken, placed in a sterile petri dish and then dropped with 0.1 mL of fluorescent pseudomonad suspension for 1 minute. Then the disc is placed in the middle of the medium which has been inoculated with BDB suspension. Observations were made after 2x24 hours of incubation by calculating the amount of inhibition zone formed using calipers.

3. Results
3.1. The Production of Siderophore
All observed fluorescent pseudomonads have different abilities in producing siderophores. Siderophore levels were determined using a spectrophotometer at a wavelength of 410 nm, and the resulting number was a number of Optical Density (OD). The highest siderophore production was 1,027 (OD 410 nm), produced by the fluorescent pseudomonad PfLAHp2, and the lowest was produced by PfCas at 0.148 as shown in Table 1.

| Fluorescent pseudomonad | Siderophore (OD 410 nm) |
|------------------------|------------------------|
| PfLAHp2                | 1,027                  |
| PfPj1                  | 0,992                  |
| PfKd7                  | 0,892                  |
| PfPj2                  | 0,670                  |
| PfPb1                  | 0,633                  |
| PfCas3                 | 0,533                  |
| PfCas                  | 0,148                  |

3.2. Fluorescent Pseudomonad Antagonist Test Against BDB
Antagonistic tests on BDB were performed for each siderophore-producing fluorescent pseudomonad. Antagonistic ability is characterized by the formation of inhibitory zones. The results showed that the fluorescent pseudomonad PfLAHp2 was significantly different from the other 6 isolates, and had the highest antagonistic ability against BDB, which was 1,042 cm. While the lowest antagonistic ability is found in fluorescent pseudomonad PfCas, as shown in Table 2.

| Fluorescent pseudomonad | Diameter of inhibitory zone (cm) |
|------------------------|----------------------------------|
| PfLAHp2                | 1,042 a                          |
| PfPj1                  | 0,974 b                          |
| PfKd7                  | 0,845 c                          |
| PfPj2                  | 0,657 d                          |
| PfPb1                  | 0,616 d                          |
| PfCas3                 | 0,552 e                          |
| PfCas                  | 0,249 f                          |

4. Discussion
This research shows that all fluorescent pseudomonad isolates used can produce siderophores. However, each isolate produced a different number of siderophores (Table 1.). According to Khan et al., [11] variations in the production siderophore of a bacteria, depends on space, time, environment and origin of the organism itself. Added by Kumar et al., [12], nutritional differences can also affect siderophor production. Advinda et al., [13] reported that various mineral sources added to the
All fluorescent pseudomonad isolates tested had different antagonistic abilities against BDB. The fluorescent pseudomonad PfLAHp2 has the highest antagonistic ability against BDB, while the lowest antagonistic ability is produced by PfCas. The difference in antagonistic ability might be due to the production of different siderophores from each isolate. According to Beneduzi et al. [14], fluorescent pseudomonad can produce various secondary metabolite compounds. Secondary metabolites in the form of siderophores and antimicrobials are effective in protecting plant growth from phytopathogens.

Siderophores are produced by Bacillus subtilis CTS-G24, and act as a biocontrol agent against Fusarium oxysporum f. sp. ciceri and Macrophomina phaseolina, phytopathogenic bean plants (*Cicer arietinum*) [15]. According to Prema and Selvarani [16], Pseudomonas aeruginosa was found to be efficient in producing siderophores and used as a control for pathogenic fungi. The resulting siderophore can be antagonistic to the pathogenic fungus *F. oxysporum* and *Sclerotium rolfsii*.

5. Conclusion
From the research it was concluded that fluorescent pseudomonad isolates PfPj1, PfPb1, PfPj2, PfKd7, PfCas, PfCas3, and PfLAHp2 can produce siderophore. The PfLAHP2 isolate produced the highest siderophore 1.027, and the lowest PfCas isolate was 0.148. Fluorescent pseudomonad antagonist test on BDB showed that PfLAHP2 isolate produced the largest inhibitory zone, which is 1.042 cm.

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