Potential of *Bacillus spp* produces siderophores in suppressing the wilt disease of banana plants

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**Abstract.** In nature, different types of siderophore such as hydroxymate, catecholets and carboxylate, are produced by different bacteria. *Bacillus spp* were isolated from potato rhizospheric soil can produce siderophore of both catecholets and salicylate type with different concentrations. Various strains of *Bacillus spp* were tested for pathogen inhibition capability in a dual culture manner. The test results showed the ability of inhibition of pathogen isolated from banana wilt disease. From the result tested were found *Bacillus niabensis* Strain PT-32-1, *Bacillus subtilis* Strain SWI16b, *Bacillus subtilis* Strain HPC21, *Bacillus mojavensis* Strain JCEN3, and *Bacillus subtilis* Strain HPC24 showed different capabilities in suppressing pathogen.

**Keywords:** *Bacillus spp*, wilt disease, banana

1. **Introduction**

Current research on microbial siderophore in the rhizosphere is associated with their biocontrol activities due to their competitive effects with plant pathogens. Bacteria produce a large variety of siderophores under iron-limiting conditions: hydroxamates, phenol-catecholates, and carboxylates [2]. In nature, different types of siderophore such as hydroxymate, catecholets and carboxylate, are produced by different bacteria. Hydroxymate siderophore possess N-hydroxylated amide bonds as co-ordination sites, catecholates co-ordinate iron with catecholate hydroxyl group and carboxylates co-ordinate iron with carboxyl and hydroxyl groups [1]. Siderophore produced by rhizospheric bacteria improve rhizosphere colonization and play an important role in iron mineralization & supplement to plant. It has been suggested that siderophores are antagonistic by means of sequestering iron from the environment, restricting growth of the pathogen [2-4]. Siderophores facilitate the solubilization and transport of iron into the cell by cognate transport system. Siderophores efficiently deplete iron from the environment making it less available to certain competing microorganisms including plant pathogens.

*Bacillus* are most predominant colonies in the rhizosphere, potential general due to their spore forming ability [5-7] can elicit plant defenses suppresses the plant pathogens by producing siderophore compounds also induces the Induced systemic resistance (ISR) in host plant and reduces the incidence of disease severity in host plants against pathogens [8]. The objectives of this study were to know the potential of *Bacillus* in producing siderophores to suppress wilt disease in bananas plant.
2. Materials and methods

2.1 Source of Bacillus spp
The Bacillus species used are collection of Physiology Laboratories of Agriculture Faculty-Pattimura University which has been identified molecularly based on gen sequence of 16S rRNA and phylogenetic [9].

2.2 Estimated of siderophores
The production of bacterial isolate siderephores was tested as follow, The media used is media NB. One ml of bacterial isolate was added to each flask and incubated at 37ºC for 7 days. After seven days of incubation, culture of bacterial isolate was centrifuged at 10,000 g for 20 min. Supernatant is used to estimate siderophores salicylate type. Twenty ml of supernatant culture was taken and the pH was set to 2.0 with HCl solution. For 20 ml of supernatant was added 20 ml of ethyl acetate and extraction twice. Five ml of the test solution were added with 5 ml of the Hathway reagent (1 ml of 0.1 M ferric chloride and 1 ml of 0.1 N HCl added to 100 ml of distilled water and 1 ml of 0.1 M potassium ferricyanide added) and the absorbance was measured at 560 nm with sodium salicylate as a standard for salicylate estimation. Standard sodium salicylate is prepared from dilution with a concentration of salicylate sodium ranging from 0 to 2 mg l⁻¹. To measure the concentration of catechol type siderophores, five ml of the test solution was added with five ml of Hathway reagents and absorbance was determined at 700 nm with 2.3 DHBA as standard. The concentration in the culture filtrate was determined and expressed as mg l⁻¹.

2.3 Confirmation of infected plant samples
Crop samples obtained from farmers' gardens from Central Ceram Manucipal, Maluku Province affected by wilt disease in banana plants were collected for isolation. The infected tissue shows a milky white strand composed of bacterial mass, which seeps out of the infected parts of the crop after being placed in a water-filled test tube.

2.4 Isolation of Pathogenic Bacteria
The source of bacterial inoculum was taken from banana plant from Tial village of Ambon Island, which showed symptoms of bacterial wilt disease allegedly caused by Ralstonia solanacearum infection with characteristic of wilting on leaves on old plants and yellowing leaves followed by necrosis. The skin looks normal, some look yellow and black. The inside of the fruit looks reddish brown and slimy mucus. The infected stem part of the plant is removed with a sterile scalpel, were small pieces are placed in distilled water for 10 to 15 minutes. The inoculation circle is immersed in the liquid and cultured on the NA medium.

2.5 Biovar determination
Procedure determination of biovar preceded with test uses mineral medium (NH₄H₂PO₄ 1.0g, KCl 0.2g, MgSO₄.7H₂O 0.2g, Difco bacto peptone 1.0g, Agar 3.0g and bromothymol blue 80.0 mg per liter) containing 1% sugar. Test biovar based on bacterial abilities utilizing sucrose, lactose, maltose and manitol, sorbitol dan dulcitol were performed following procedure of [10, 11]. A total of 200 μl of liquid media is inserted into microtiter. Suspension of pathogenic bacterial isolate inoculum with a concentration of about 10⁸ CFU / ml was prepared and cultured for 24-48 hours in sterile aquades. A total of 20 μl of bacterial suspension was added to microtiter plate wells and incubated at 28-32°C. After 3 days of inoculation was observed discoloration [12].

2.6 Test of inhibition zones in some media
Inhibition zone formation test using several media such as: NA; King’s B Agar; YPDA. 9 ml Bacillus spp isolates from medium NB was added with 1 ml suspension of pathogenic bacteria into a petridish. Filter paper with a diameter 5 mm dipped into Bacillus spp bacterial suspension from various strains is placed in the center of the agar surface. As a control, the piece of filter paper is im-
mersed in a sterile aquadest. Each strain of Bacillus spp was tested three times and the inhibition zone diameter was measured.

3. Results and discussion

3.1 Siderophores testing
Microbes capable of producing siderophores may affect the biocontrol, virulence and availability of iron nutrients for plants [13,14,15,16]. Rizosphere bacteria can produce various siderophores. Generally, siderophores bacteria contain catecholates, and some also contain carboxylates and hydroxamates [17]. The results of this research by testing the production of catechol and salicylate showed that the rhizosphere bacteria from the potato plant produced the species of catechol and salicylate siderophores with different concentration (Table 1).

Table 1. Siderophores Production Capability by Some Strain Bacillus.

| Bacteria [18]                  | Type of Siderophore | Catechol (mg l⁻¹) | Salicylate (mg l⁻¹) |
|--------------------------------|---------------------|-------------------|--------------------|
| Bacillus niabensis Strain PT-32-1 |                      | 2.26              | 3.23               |
| Bacillus subtilis Strain SW116b   |                      | 3.35              | 1.71               |
| Bacillus subtilis Strain HPC21    |                      | 2.87              | 4.21               |
| Bacillus mojavensis Strain JCEN3  |                      | 4.21              | 4.12               |
| Bacillus subtilis Strain HPC24    |                      | 3.03              | 2.77               |

3.2 Isolation of pathogenic bacteria
Result of isolation pathogenic bacteria from banana stem showing symptom of wilt. Isolate pathogenic bacteria grown in milk color and including gram-negative bacteria characterized by round colony, white color and no slime after detection with 3% KOH.

3.3 Biovar determination
The race classification, biovar and phylotype generally aims to divide R. solanacearum species. Racial pattern system that classifies R. solanacearum strains according to the ability to infect host plants. The results of biovar determination of pathogenic bacterial causes of wilt disease in banana plants including biovar 3. This type of bacteria is able to oxidize carbohydrates (sucrose, lactose, dextrose and cellobiose) and sugar alcohol (mannitol and dulcitol) which is indicated by color changes according to Table 2 and Figure 1.

Table 2. Determination of Bacterial Pathogenic Biovar on Banana Plant Disease.

| Useful | Biovar | 1 | 2 | 3 | 4 | 5 |
|--------|--------|---|---|---|---|---|
| Sukrose|        | - | + | + | - | + |
| Lactose|        | - | + | + | - | + |
| Dextrose|       | - | + | + | - | + |
| Cellobiose|   | - | + | + | - | + |
| Mannitol|       | - | - | + | + | - |
| Dulcitol|       | - | - | + | + | - |
3.4 *In vitro antagonists against bacterial disease on banana plants*

In vitro testing to inhibit bacterial wilt diseases from banana plants found that all of *Bacillus spp* strains tried to control wilt disease in banana plants. This is indicated by the formation of halozone around the bacteria tested (Figure 2). The results of antagonistic test showed the presence of inhibitory activity by *Bacillus spp* on the pathogen of wilt disease in banana plants. The difference in inhibition by each strain of *Bacillus spp* is shown in Table 3. *Bacillus spp* is antagonistic to various plant pathogens [19,20,21] including pathogens of wilt banana plants.

![Figure 2. Halozone: a) Bacillus mojavensis Strain JCEN3 in Nutrien Medium, b) Bacillus subtilis Strain HPC2-1 in Bacillus subtilis Strain HPC2-1 Yest Peptone Dextros Agar (YPDA), and c. Bacillus niabensis Strain PT-32-1 in Kings B Medium.](image)
Table 3. Halozone Formation by Bacillus spp on Some Types of Media Against Bacterial Pathogens
Causes of Banana Disease in Banana Plants

| Strain Bacillus spp [18] | Halozone Diameter (mm) |
|--------------------------|------------------------|
|                          | Nutrien Agar           | Yest Pepton Dekstrose Agar | Kings B |
| *Bacillus niabensis* Strain PT-32-1 | 10                     | 8.5                        | 8.5     |
| *Bacillus subtilis* Strain SWT16b     | 10.5                   | 6.5                        | 7.5     |
| *Bacillus subtilis* Strain HPC21      | 9.5                    | 6.5                        | 7.5     |
| *Bacillus mojavensis* Strain JCEN3    | 9.5                    | 6                          | 6       |
| *Bacillus subtilis* Strain HPC24      | 8.5                    | 10.6                       | 6       |

4 Conclusion
Bacteria causing wilt diseases in banana plants belonging to biovar 3 which is indicated by the color change on through the biovar determination test. *Bacillus spp* strains tested in vitro are able to control wilt disease in banana plants, which is shown by the formation of halozone around the bacteria.

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