Screening of Rizoplan Rhizobacteria for Suppression of Bacterial Wilt (*Ralstonia solanacearum*) and Promoting the Growth on Chili (*Capsicum annum*)

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Abstract. *Ralstonia solanacearum* (Rs) is a pathogen that causes bacterial wilt on chili. Rs infected more than two hundred crops and caused high impact losses. The methods in controlling of Rs by using bactericide which are harmful to the environment. Therefore, it is necessary to control bacterial wilt disease effectively and efficiently by utilizing rhizobacteria rizoplan as biological agents. The aim of this study was to obtain rhizobacteria that colonize root in preventing the entry of Rs through root and stimulating the growth of chili. This experiment consisted of two stages, (i) screening of rhizobacteria isolates and (ii) introduction of selected isolates in suppressing Rs and promoting growth of chili. The research was conducted in randomized block design with 21 treatments with 5 replications for each treatment. Chili seeds were introduced with rhizobacteria isolates before planting. The results showed that there were isolates that were able to increase plant growth, Rp. Han-1.4; Rp. Han-9.2; Rp. Han-6.2; Rp. Han-1.4; 5.2 and Rp. Han-9.1 with effectiveness of 69.20% - 75% (plant height) and 57.29-59.22% (number of leaves). The isolates that were able to suppress disease progression were Rp. Han-1.4; Rp. Han-9.2 and Rp. Han-3.2 with an effectiveness of 44.99 - 53.75% (disease incidence) and 32.09-44.95% (disease intensity).

1. Introduction

Chili is a plant that has economic value and high nutritional content. One of the fluctuations in the productivity of chili is caused by bacterial wilt caused by *Ralstonia solanacearum* (Rs). Rs which is pathogenic bacteria that causes wilt and is ranked as number two bacteria in the list of important bacteria in causing disease in various plant species [1]. Rs has wide host range and it is reported that more than 200 plants can become infected and cause economic loss in the world [2]. The high attention to the negative impact of using bactericides requires a control method that is friendly to non-target organisms, the environment and does not cause pathogen resistance, namely by utilizing biological agents [3].

Rizoplan is biological agent of the rhizobacterial group on the surface of plant roots and has been reported to be able to control various diseases caused by plant pathogenic bacteria [4]. Plants introduced with rhizobacteria sp can increase the height in tomato plants infected by Rs [5]. *Bacillus megaterium* B1301 can reduce the intensity of bacterial wilt caused by Rs in ginger with an effectiveness of 73.68% [6].
Research on the use of biological agents using rhizobacteria is increasing. Rhizobacteria have great potential in controlling bacterial wilt disease in various plants [7] including chili and are expected to reduce the use of bactericides that have many negative effects. Rizobacteria can also be used and reproduced by the community. Therefore, the aim of this study was to obtain rhizobacteria that colonize root in preventing the entry of Rs through root and stimulating the growth of chili.

2. Materials and Method

2.1. Place and Time
This research was conducted in the Laboratory of Basic Science and Plant Protection and screen house, Department of Agroecotecchnology, Faculty of Agriculture, University of Sultan Ageng Tirtayasa from September to November 2019.

2.2. Materials and Method
The materials used in this study were: chili seeds, *Mirabilis jalapa*, rhizoplan bacterial isolates, Nutrient Agar (NA) medium, Kelman’S TZC Agar, 70% alcohol, distilled water, heat-resistant plastic bags, cotton, manure, artificial fertilizers, aluminum foil, tissue, plastic wrapping, plastic ropes, polybags, glass of mineral water vol. 240 ml, marker, filter paper, and label paper. The tools used were: glass Petri dishes, microtube, test tubes, goblets, measuring cups, Erlenmeyer flasks, laminar air flow cabinets, magnetic stirrers, autoclaves, ovens, hot plates, vortices, digital scales, Schoot bottles, glass objects, pipettes micro, pipettes, test tube racks, porcelain mortar and mortar, stirring rods, spiritus lamps, loop needles, syringes, pins, incubation rooms, buckets. This research used a method which was used in screening and introducing rhizobacteria in Chilli rhizoplane [3].

2.3. Research Design
This study used a randomized block design with 21 treatments. The treatment was a combination of the introduced isolates. Each treatment and control combination was repeated 5 times so that there were 106 experimental units. Chili seeds were introduced with rhizobacteria isolates before planting. The data were analyzed using variance, if they were significantly different, then continued with Duncan's New Multiple Range Test (DNMRT) at the 5% level.

2.4. Isolation of Rhizobacteria and Rs
The samples of chili plants came from Kadupayung and Muruy Villages, Menes District, Pandeglang Regency, Banten Province. Rizoplan was taken from chili root samples 1.5-2 months old, healthy, and had good growth. Soil, root and plant samples were put in plastic bags, taken to the laboratory and stored in an AC room at 20°C. Isolation of rhizobacteria was using the serial dilution method [8]. The root sample was weighed 1 g, put in 10 ml of sterile distilled water in a test tube and diluted to $10^{-6}$. The suspensions from $10^{-6}$ and $10^{-7}$ dilutions were transferred 0.07 ml into a test tube containing 9 ml of liquid NA medium, homogenized with vortex, poured into a petridish, and incubated for 3 × 24 hours at room temperature. Isolates were purified by streak method on NA media in a petridish and incubated for 2 × 24 hours. Pure isolates were transferred with a loop needle into a microtube containing 0.5 mL of sterile sodium hypochlorite. Rs was isolated from chili plants showing wilting symptoms using the same method and grown on Kelman's TZC agar medium and incubated for 48 hours.
2.5. Rejuvenation and Propagation of Rhizobacterial Isolates

In the first stage, Rhizobacteria in microtube were rejuvenated using the strike method on Nutrient Agar (NA) medium and incubated for 48 hours. Rhizobacteria propagation consisted of two steps: First, for the preculture of 1 single rhizobacteria colony puts into 50 mL of Nutrient Broth (NB) medium and incubated in a horizontal shaker for 24 hours and 150 rpm. Second, for mainculture 1 mL of the preculture was transferred to 99 mL of NB in an Erlenmeyer flask (vol. 250 mL) and incubated in a horizontal shaker for 48 hours. The population density of rhizobacterial suspension from mainculture was determined by calculating the turbidity of rhizobacterial suspension using a spectrophotometer with a wavelength of 600 nm.
2.6. Rs Pathogenicity and Isolates Hypersensitive (HR) Test
The pathogenicity test was carried out by inoculating Rs by injuring the chili stems and attaching them to cotton that had been soaked in Rs suspension and observing the progress of the disease. HR test was carried out by injecting a suspension of rhizobacteria isolates into the intercellular space in the *Mirabilis jalapa* leaf tissue and observed symptoms appearing 48 hours. If necrotic symptoms did not appear, the isolates were used for morphological observations (color, shape, colony edges) and physiological characteristics (Gram test). Rhizobacteria suspension and Rs in the pathogenicity test and HR test were measured to reach a density level of up to 108 CFU.mL⁻¹ using a spectrophotometer with a wavelength of 600 nm.

**Table 1.** Morphological characters of Rizoplan isolates and physiology on chili roots

| Rhizoplane Isolates | Colony Color | Optic Characteristic | Form | Margin | Elevation | Radius (mm) | Gram * | HR** |
|---------------------|--------------|----------------------|------|--------|-----------|-------------|--------|------|
| Rp.Han-1.1          | White        | Transculeate         | Round| Entire | Raised    | 1.91        | -      | -    |
| Rp.Han-1.2          | Kuning       | Opaque               | Irregular | Filamentous | Convex | 1.45        | +      | -    |
| Rp.Han-1.3          | White        | Opaque               | Rhizoid | Undulate | Umbonate | 3.02        | -      | -    |
| Rp.Han-1.4          | White        | Opaque               | Rhizoid | Round | Filamentous | Datar | 1.64    | +    | -    |
| Rp.Han-2.1          | White        | Transculeate         | Round| Undulate | Umbonate | 3.85        | -      | -    |
| Rp.Han-2.2          | White        | Opaque               | Round | Filamentous | Raised | 2.42        | -      | -    |
| Rp.Han-3.1          | White        | Opaque               | Round | Filamentous | Umbonate | 2.55        | -      | -    |
| Rp.Han-3.2          | White        | Transculeate         | Rhizoid | Entire | Umbonate | 2.04        | -      | -    |
| Rp.Han-4.1          | White        | Opaque               | Round | Undulate | Umbonate | 4.13        | +      | -    |
| Rp.Han-4.2          | Transparenent| Transparenant        | Rhizoid | Serrate | Umbonate | 1.43        | +      | -    |
| Rp.Han-5.1          | White        | Transculeate         | Irregular | Entire | Convex | 2.74        | -      | -    |
| Rp.Han-5.2          | Kuning       | Opaque               | Round | Lobate | Umbonate | 2.58        | +      | -    |
| Rp.Han-6.1          | White        | Opaque               | Irregular | Entire | Umbonate | 2.31        | +      | -    |
| Rp.Han-6.2          | White        | Transculeate         | Rhizoid | Serrate | Convex | 3.55        | +      | -    |
| Rp.Han-7.1          | Transparent  | Transparenant        | Rhizoid | Filamentous | Raised | 2.34        | -      | -    |
| Rp.Han-8.1          | White        | Transculeate         | Rhizoid | Filamentous | Umbonate | 2.96        | -      | -    |
| Rp.Han-8.2          | White        | Opaque               | Irregular | Lobate | Umbonate | 1.90        | -      | -    |
| Rp.Han-9.1          | White        | Transculeate         | Irregular | Filamentous | Umbonate | 2.34        | -      | -    |
| Rp.Han-9.2          | Transparent  | Transparenant        | Rhizoid | Filamentous | Datar | 2.27        | +      | -    |
| Rp.Han-9.3          | Transparent  | Transparenant        | Rhizoid | Filamentous | Raised | 2.43        | +      | -    |
| Rp.Han-9.4          | White        | Transculeate         | Rhizoid | Filamentous | Umbonate | 2.73        | -      | -    |
| Rp.Han-9.5          | White        | Transculeate         | Rhizoid | Filamentous | Raised | 1.88        | +      | -    |

Note: * (+) = Gram Positive, (-) = Gram Negative
** (+) = HR Positive, (-) = HR Negative

2.7. Introduction of Selected Isolates in Promoting Growth and Suppressing Bacterial Wilt on Chili
This research was conducted with an experimental method using a randomized block design in a screen house in the Agroecotechnology Department, Faculty of Agriculture, Sultan Ageng Tirtayasa University. The isolates used were selected isolates with a negative HR test result. The treatment consisted of two factors, namely selected rhizobacteria isolates and controls. In each treatment there were 5 replications. The selected rhizobacteria isolates were rejuvenated and propagated using the technique in the first stage. Rhizobacteria population density of 108 CFU/mL⁻¹ was calculated using a spectrophotometer with a wavelength of 600 nm. The chilli seeds were soaked in rhizobacteria
suspension for 30 minutes and planted at a depth of 1-2 cm from the soil surface and covered with a layer of soil on polybags. A suspension of Rs (108 CFU / mL) was inoculated by injecting it into the stem of a 1 month old chili plant. The chili seeds used were first washed with sterile distilled water and sterilized with 1% NaOCl solution for 3 minutes, then rinsed twice with sterile distilled water. The chili seeds without treatment (control) were directly planted in polybags. Sources of chili seeds came from Kadupayung and Muruy Villages, Menes District, Pandeglang Regency, Banten Province. The observation included bacterial wilt disease progress (incubation period, disease percentage and intensity, number of leaves, and plant height).

3. Results and Discussion

3.1. Plant height
Generally, chili height increased in plants introduced by rizoplan isolates. There were seven isolates that were significantly different than the control. The plant height that rizoplan introduced was 30.10 - 52.02 cm with an effective plant height of 24.89 - 59.30%. Early observation, the plant height looked the same and at week 3 there was a difference between treatment and control and it continued to increase until the end of the observation (Figure 3). Seven rizoplan isolates were able to increase plant height with an effectiveness of more than 50% (Table 2). The rhizoplan isolates included: Rp. Han-9.2 (52.02 cm and an effectiveness of 57.27%), Rp.Han-1.4 (51.98 cm and effectiveness of 57.23%), Rp.Han-6.2 (51.48 cm and an effectiveness of 56.82%), and Rp.-5.2 (51.02 cm and effectiveness of 56.43%), Rp.Han-9.1 (49.18 cm and effectiveness of 54.80%), Rp.Han-8.2 (47.55 cm and effectiveness of 53.25%) and Rp.Han-7.1 (46.48 cm and effectiveness of 52.17%).

![Plant Height graph](image)

**Figure 3.** The progress of chili height introduced by rhizoplane isolate at 1 - 7 weeks after planting (WAP).

3.2. Leaves Number
The number of leaves on chili increased in plants introduced by rhizoplan. Early observation (1 mst) to the end of the observation (7 mst), which ranged from 18.17 to 51.33 with the effectiveness ranging from 51.33 - 29.36%. At 1-4 mst, the number of leaves was relatively the same between treatment and control. The difference in the number of leaves began to appear at 5 mst of plants introduced by rhizoplan isolates and continued to increase with increasing plant age (Figure 4). The four best rhizoplan isolates that were able to increase the number of leaves by more than 70% were: Rp. Han-
1.4 (51.33 strands and 75% effectiveness), Rp. Han-9.2 (50.50 strands and 74.59% effectiveness), Rp. Han-6.2 (44.83 strands and 71.38% effectiveness), Rp. Han-5.2 (44.33 pieces and 71.05% effectiveness).

![Figure 4](image-url)  
**Figure 4.** The progress of the number of chili leaves introduced by rhizoplan isolates at 1 - 7 weeks after planting (WAP)

### 3.3. Disease Progress

All chili introduced by isolates had a lower percentage and intensity of plant disease than the control. The percentage of disease in treatment was 11.41-21.44% compared to control (24.67%). There were ten isolates that were significantly different than control in the percentage of attacks. Two isolates with an effectiveness of more than 50%, namely Rp. Han-1.4 (53.75%) and Rp. Han-6.2 (50.95%). Meanwhile, the intensity of disease introduced by rizoplan ranged from 20 to control (36.33% - 33.47%). There were nine isolates that were significantly different than the control. There were two isolates with an intensity effectiveness of more than 40%, namely Rp. Han-1.4 and Rp. Han-3.2 (20%, 44.95% effectiveness) with resistance reactions in chilli plants introduced by rizoplan isolates, namely somewhat sensitive (AP) and somewhat tolerant (AT).

### Table 2. Disease development and plant growth after 7 weeks after planting introduced by rizoplane and their effectiveness.

| Rizoplan Isolates | Leaves Number | Height Plant | Disease Incidency | Disease Intensity | Reaction ** |
|-------------------|---------------|--------------|-------------------|-------------------|------------|
|                   | Leaves Number* | E (%)        | Cm*              | E (%) %*          | E (%) %*   | E (%)      |                     |
| Rp.Han-1.4        | 51.33 a       | 75.00        | 51.98 a          | 57.23             | 11.41 a    | 53.75      | 20.00 a          | 44.95              | AT         |
| Rp.Han-9.2        | 50.50 a       | 74.59        | 52.02 a          | 57.27             | 13.57 a    | 44.99      | 24.67 abc        | 32.09              | AT         |
| Rp.Han-6.2        | 44.83 ab      | 71.38        | 51.48 ab         | 56.82             | 12.14 ab   | 50.95      | 22.00 ab         | 39.44              | AT         |
| Rp.Han-5.2        | 44.33 ab      | 71.05        | 51.02 ab         | 56.43             | 12.34 ab   | 49.98      | 22.67 ab         | 37.60              | AT         |
| Rp.Han-9.1        | 41.67 ab      | 69.20        | 49.18 ab         | 54.80             | 16.55 abc  | 32.91      | 25.67 abc        | 29.34              | AT         |
| Rp.Han-1.2        | 38.17 ab      | 66.38        | 41.92 abc        | 46.97             | 16.93 abc  | 31.37      | 26.60 abc        | 26.78              | AT         |
| Rp.Han-8.2        | 36.83 ab      | 65.16        | 47.55 ab         | 53.25             | 14.45 ab   | 41.43      | 24.13 abc        | 33.58              | AT         |
| Rp.Han-7.1        | 35.67 ab      | 64.02        | 46.48 ab         | 52.17             | 14.08 ab   | 42.93      | 24.00 ab         | 33.94              | AT         |
| Rp.Han-5.1        | 33.33 ab      | 61.50        | 42.22 abc        | 47.35             | 15.04 abc  | 39.04      | 24.67 abc        | 32.09              | AT         |
The chili plants introduced by rhizoplan isolate were able to increase plant height. The highest plant height was in chili plants introduced with isolate Rp. Han-9.2 (52.02 cm) compared to control (22.23 cm). Tomato plants introduced with Methylobacterium sp could increase the height in tomato plants infected by Rs [5]. *Metilobacterium* sp is included in rhizobacteria which can increase plant growth. Rizoplan is able to increase plant height by increasing the availability of phosphorus in the soil [12], increasing plant tolerance to high soil salinity through the secretion of the ACC-Deaminase enzyme [13]. Increasing the ability of plants to absorb micro and macro elements (P, N, K, Ca and Mg) [10] and the auxin secreted Fitohormones [14].

4.3. The Progress of plant diseases

The chili plants introduced by rhizoplan were able to suppress the development of plant diseases and increase resistance to disease. This mechanism, among others, is through the formation of biofilms through the secretion of N-Acyl Homoserine Lactone (AHL), thereby increasing the ability to colonize roots [15]. The ability to suppress the development of sprouts was related to the ability of rizoplan to colonize roots and the effect of antibiosis on tomato plants [16]. Bacillus spp. was able to control the development of pathogens in *B. thuringiensis* through the production of acyl homoserine lactone (AHL) lactonases [17]. The secretion of the enzyme HL lactonases by rizoplan can hydrolyze the acyl homoserine lactone molecule thus inhibiting the quorum sensing signal in plant pathogens. Inhibition
of these molecular signals reduces the virulence factor so as to reduce the percentage of soft rot attack on potatoes [18]. Another mechanism is the secretion of the chitinase enzyme which can degrade the cell walls of pathogenic fungi [19], terpenoid antimicrobial compounds, phosphanates, bacteriocins, [20], Autolisin [21], siderophore and catechol [22].

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