# SELECTION AND CHARACTERIZATION OF SIDEROPHORE-PRODUCING RHIZOBACTERIA AND POTENTIAL ANTAGONISTIC ACTIVITY TOWARD *Ralstonia solanacearum*

ABDJAD ASIH NAWANGSIH[1*], IDA PARIDA[1], SURYO WIYONO[1] and JUANG GEMA KARTIKA[2]

[1]*Department of Plant Protection, Faculty of Agriculture, Institut Pertanian Bogor, Bogor 16680, Indonesia*
[2]*Department of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor, Bogor 16680, Indonesia*

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**ABSTRACT**

*Ralstonia solanacearum* is an important disease of tomato. An alternative method to control the disease is the application of biocontrol agents. Plant Growth-Promoting Rhizobacteria (PGPR) could be used as potential biocontrol agents. PGPR with siderophores is among compounds having important role in disease suppression. This experiment was conducted to select and characterize the siderophore-producing rhizobacteria from tomato and to determine their potential as antagonistic agents for *R. solanacearum*. Candidates of the PGPR were isolated from tomato grown in West Java Province, Indonesia. The isolates were detected as siderophore-producing bacteria using CAS medium. Among 29 isolates producing siderophore and having negative result on hypersensitivity reaction, two isolates provided the widest diameter of inhibition zone toward *R. solanacearum*. Both isolates were CP1C and CP2D with diameter of inhibition zone up to 3.6 and 7.0 mm, respectively. Based on the sequence of 16S rDNA, isolate CP1C was identified as *Brevundimonas* sp., while isolate CP2D was identified as *Enterobacter* sp. Both bacteria did not cause negative effect on the increasing plant height and dry weight of the plants, compared with control.

**Keywords**: Bacterial wilt, biocontrol, *Brevundimonas*, *Enterobacter*, PGPR

## INTRODUCTION

Bacterial wilt of tomato caused by *Ralstonia solanacearum* is one of important diseases of tomato in tropics and subtropics area (Jeung *et al.* 2007). The bacteria survive for a long time in the soil (Wang & Lin 2005). The bacteria also multiply in the xylem as well as attacking the xylem and affect the water and nutrient translocation, causing wilting and death of the plant (Agrios 2005). Among the control methods to control the disease, is the application of biological control (biocontrol) agents (Yuliar *et al.* 2015). According to Jenifer *et al.* (2013), one of the most important mechanisms responsible for suppressing *Pseudomonas* sp. (a plant pathogen) is siderophore-mediated competitions for iron.

* Corresponding author: asnawangsih@yahoo.com

Siderophores (from the Greek "iron carriers") are small ferric-ion-specific chelating agents produced by bacteria and fungi which grow in low iron condition causing them to scavenge iron from the environment and to make iron available to the microbial cell (Neilands 1995; Pal & Gokarn 2010). Siderophores are also known to bind molybdenum and lead (Pal & Gokarn 2010).

Sayyed *et al.* (2005) reported that siderophore-producing *Pseudomonas* sp. plays vital role in stimulating plant growth and in controlling several plant diseases. In *Pseudomonas fluorescens* the pigment produced is a siderophore. Pigment production is increased in the presence of sodium, potassium, lead, molybdenum, cadmium and ammonium sulphate ($(NH_4)_2SO_4$) (Bhattacharya 2010; Sayyed *et al.* 2005). Adding exogenous amino acid did not significantly increase the production of siderophore (Hu & Xu

2011). Sayyed *et al.* (2005) also reported that Mn, Hg and Co showed inhibitory effect on siderophores growth and production. Presence of potassium, magnesium and calcium had little inhibitory effect on siderophores production compared to controls (Battacharya 2010).

Sayyed *et al.* (2005) reported that production of siderophores by bacteria was affected by growing media. In growing media such as Nutrient Broth (NB) and MacKonkey's Broth (MB), siderophores were not produced because both media are luxurious media having high content of Fe. Fe-free succinic acid medium (SM) was found to provide maximum (92.25%) siderophores production in comparison to 88.00% in Barbhayya Rao broth (BR), 79.00% in Cassamino Acid broth (CAA) and 48.00% in Enrichment Medium (EM) (Sayyed *et al.* 2010). A study on pH effect to the production of siderophores by *Rhizobium* sp. showed that the growth and production of siderophores started at pH 4.5, reaching maximum at neutral pH; at pH 10.0, there was no siderophores production (Sridevi *et al.* 2008). Study on genetic diversity of tobacco rhizosphere which produces siderophore demonstrated that 85% of the total 354 isolates produced siderophores in iron limited liquid medium; some of them are *Pseudomonas*, *Enterobacter*, *Serratia*, *Pantoea*, *Erwinia* and *Stenotrophomonas* which belong to γ-*Proteobacteria* (Tian *et al.* 2009).

This experiment was conducted to select and characterize the siderophore-producing rhizobacteria from tomato and to determine their potential as antagonistic agents for *R. solanacearum*.

## MATERIALS AND METHODS

### Isolation and Quantification of Siderophore-producing Rhizobacteria

Siderophore-producing rhizobacteria were isolated from rhizosphere samples taken from healthy tomato plants, collected from tomato field in Cipanas Sub-district (Cianjur District) and Lembang Sub-district (West-Bandung District). In Cipanas Sub-district, samples were collected from three villages, while in Lembang Sub-district samples were collected from two villages. Both sub-districts are the center of tomato field in West Java Province, Indonesia. From each village, 1,500 g of rhizosphere soil samples (with tomato roots) were collected from five different plots (300 g per plot as subsample) and thoroughly mixed using trowel, until becoming a composite sample. From each composite sample, 10 g of rhizosphere soil was immersed in 90 mL 0.85% NaCl. After a serial dilution, 0.1 mL of each dilution was inoculated on Chrome Azurol Sulphate (CAS) medium (Gross 1990; Louden *et al.* 2011). Inoculation was repeated three times. Plates containing the inoculation were incubated at room temperature (±28 °C). Numbers of colony of siderophore-producing rhizobacteria were calculated at 24 - 48 hours after incubation, based on the production of orange zone around colony of the bacteria. Successfully isolated bacteria were transferred to King's B agar medium and separated from each other to obtain pure culture. Each isolate was preserved in Nutrient Broth (NB) with 20% glycerol and kept in -20 °C environment. For daily maintenance, isolates were preserved in NB and kept at room temperature (±28 °C).

### Hypersensitive Reaction Test

Hypersensitive Reaction (HR) test was conducted to select the nonpathogenic bacteria as candidates of biocontrol agents. The test was conducted by inoculating $10^8$ – $10^9$ cfu/mL of rhizobacteria on tobacco leaves. One milliliter of suspension was injected into tobacco leaf using 5 mL sterile syringe. Successful injection was indicated by water soaked zone around the point of injection. Inoculation of each isolate was conducted in duplo. Inoculated leaves were incubated at room temperature (±28 °C) for 24 hours. Leaves showing necrotic symptoms before 24 hours were counted as having positive reaction to the hypersensitive reaction and thus, be eliminated.

### Antagonistic Activities of Siderophore-producing Rhizobacteria against *In vitro Ralstonia solanacearum*

Bacteria isolates having negative reaction on the previous hypersensitive test were tested on their antagonistic activity toward *R. solanacearum*. Both bacteria, i.e. *R. solanacearum* and rhizobacteria, were grown on a King's B agar

plate. Five hundred micro liter suspension of *R. solanacearum* ($10^8$ - $10^9$ cfu/mL) was spread on the surface of the King's B agar plate. After being air dried, three sterilized filter papers having 0.5 cm diameter were placed besides the agar plate with 2 cm distance from each other. Filter paper in the center was inoculated with 50 µL of sterilized distilled water and designated as control. The two other filter papers were each inoculated with 50 µL suspension of one isolate of rhizobacteria. Each treatment was repeated three times. Inoculated plates were incubated at room temperature (±28 °C). The antagonistic activity was observed at 24 hours after incubation. Antagonistic activity was indicated by the production of inhibition zone around the filter paper inoculated with siderophore-producing rhizobacteria.

## Effect of Siderophore-producing Rhizobacteria on the Viability of Tomato Seeds

Tomato seeds (Arthaloka and Ratna varieties) were dipped in $10^7$ - $10^8$ cfu/mL suspension of siderophore-producing rhizobacteria for 16 hours before being planted on sterilized mixture of soil and compost (1 : 1 ratio). The soil mixture was put in 30 - 50 cm polyethylene pot tray having 128 holes. The experiment was arranged as completely randomized factorial design with 7 isolates of biocontrol agents and one control (without bacteria) as the first factor and two tomato varieties (Arthaloka and Ratna) as the second factor. Thus, 16 treatments were applied in this experiment. Each treatment was replicated three times. There were 48 units in total; each unit contained 15 seeds. One tomato seed was grown in one hole of polyethylene pot tray. The isolates of biocontrol agents used in this experiment were CP1C (code of one isolate of bacteria), CP2B, CP2D, CP3E, LB1A, LB1C and LB1L. Seeds dipped in sterilized distilled water were used as control. Layout of the treatments was shown in Table 1.

Total of the emerging seedlings were calculated every day. Seed viability (SV) was calculated using the following formula:

$$SV\ (\%) = \frac{\text{Total normal seedlings}}{\text{Total seeds sown}} \times 100\%$$

## Effect of Siderophore-producing Rhizobacteria on the Height, Fresh and Dry Weight of Tomato Plants

An experiment to test the effect of siderophore-producing rhizobacteria toward the height, fresh and dry weight of tomato plants was conducted in a green house. Tomato seeds of Arthaloka and Ratna varieties were dipped in suspension of siderophore-producing rhizobacteria ($10^7$ - $10^8$ cfu/mL) for 16 hours before being planted in 10 x 15 cm polybag filled with 2.5 – 3 kg of sterilized mixture of soil and compost (1 : 1 ratio). The experimental design applied was completely randomized factorial design having similar layout with the one presented in Table 1. The only difference was that each unit contained 5 seeds. Plant height was measured every 5 days starting from the day when the first leaf was fully opened. Total area under height of plant growth curve (AUHPGC) was calculated using

Table 1 Layout of experiment to test the effect of siderophore-producing rhizobacteria toward the viability of tomato seeds

| Tomato Varieties | | Isolate's code | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CP1C | CP2B | CP2D | CP3E | LB1A | LB1C | LB1L | Control |
| Arthaloka | Repl 1 | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds |
| | Repl 2 | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds |
| | Repl 3 | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds |
| Ratna | Repl 1 | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds |
| | Repl 2 | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds |
| | Repl 3 | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds | 15 seeds |

modification of formula reported by Van der Plank (1963):
where:

$$\text{AUHPGC} \quad \sum_{i}^{n-1} \left( \frac{y_i + y_i + 1}{2} \right) (t_{i+1} - t_i)$$

$y_{i+1}$ = increasing of plant height at the next observation
$y_i$ = increasing of plant height at the time of observation
$t_{i+1}$ = the next observation (II, III, …, VI)
$t_i$ = time of observation (I, II, …, V)

Two months after planting, all plants (five plants) from each replication of each treatment were rooted and weighted using digital balance. The average of the five plants represented data of fresh weight for each replication. Dry weight of plant was determined by drying the fresh plants in the oven at 100 °C. The weight of the sample was checked periodically until the weight of the sample was constant.

**Characterization and Identification of the Siderophore-producing Rhizobacteria**

Seven isolates of the siderophore-producing rhizobacteria were characterized based on microscopic and colony appearances, as well as on physiological and biochemical properties, following the methods of Klement *et al.* (1990) and Schaad *et al.* (2001). Two isolates of the siderophore-producing bacteria having potential as biocontrol agents were genetically identified by sequencing the 16S rDNA gene. DNA was extracted from log phase culture using phenolchloroform extraction procedure (Sambrook & Russel 2001). The 16S rDNA gene was amplified using universal primer for prokaryotes which were the forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1492R (5'GGTTACCTTACG-ACTT-3'). Total volume reaction for Polymerase Chain Reaction (PCR) was 25 µL consisted of 1 µL of DNA template; 12.5 µL of 1x Ready mix PCR, 1.5 mM $MgCl_2$, 0.2 mM dNTPs and Taq polymerase 5 units/reaction; 9.5 µL of $ddH_2O$; 1 µL of 120 pmol Primer 27F; and 1 µL of 120 pmol Primer 1492R. PCR was performed under the following conditions: one cycle of pre-denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 1 minute, annealing at 55 °C for 1 minute and extension at 72 °C for 2 minutes. The reaction was terminated with a final extension at 72 °C for 10 minutes. The PCR products were sent to the First Base Laboratory, Malaysia for sequencing. BLAST searches were performed for sequences obtained to find the similarity with sequence data in GeneBank.

**Data Analysis**

Data of the bacterial population was analyzed using t test of Minitab program version 13.3.

Effects of the bacteria to the plant growth were statistically analyzed using ANOVA for completely randomized factorial design with isolates of biocontrol agents as the first factor and tomato varieties as the second factor. Treatment means were compared using the DMRT test at 5% level of significance. SAS Program version 9.1 was used for performing statistical analyses for completely randomized factorial design and for the DMRT test.

## RESULTS AND DISCUSSION

**Abundance and Antagonistic Activities of Siderophore-producing Bacteria**

Based on the t test, the average of siderophore-producing bacteria isolated from Cipanas District was 1.98 $10^7$ cfu/g, which was not significantly different with those from Lembang District having average of 5.3 x $10^7$ cfu/g. Colonies of the siderophore-producing bacteria on CAS medium were shown in Figure 1. Siderophore production was indicated by the orange color around the colony of the bacteria. Figure 1 shows that each colony produced different amount of siderophores, indicated by the diameter of orange area around each colony of bacteria.

Among the 60 isolates of siderophore-producing rhizobacteria, 31 isolates positively showed hypersensitive reaction on tobacco, while 29 others showed negative reaction. Bacteria were also tested further for their antagonistic activities against *Ralstonia solanacearum*. Based on the antagonistic test, 16 isolates positively produced inhibition zone (Fig. 2) having diameter between 0.5 to 7.0 mm. Among those 16 isolates, 9 isolates were HR positive which had to be eliminated from being candidate of biocontrol agents. The

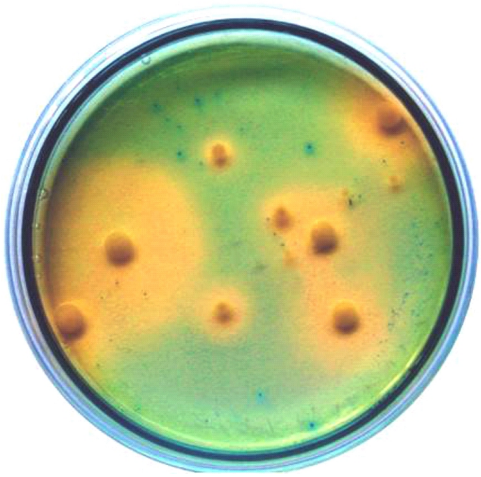

Figure 1 Production of siderophores by tomato rhizobacteria on CAS agar was indicated by yellow-orange color around colony of bacteria

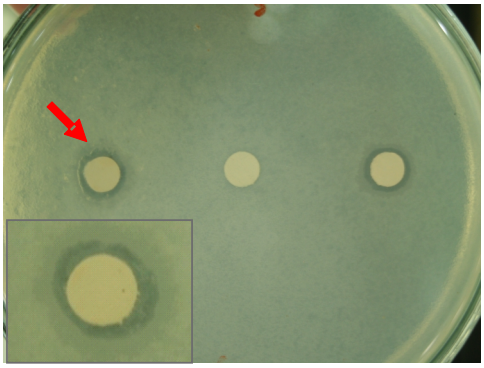

Figure 2 Production of inhibition zone (arrow sign) by the isolate of siderophore-producing rhizobacteria (Note: The inset shows magnification of the inhibition zone)

other 7 isolates were HR negative, having respective diameter of the inhibition zone of CP1C (3.6 mm), CP2B (2.3 mm), CP2D (7.0 mm), CP3E (5.0 mm), LB1A (1.6 mm), LB1C (1.6 mm) and LB1L (0.5 mm). The widest diameter of inhibition zone was produced by isolate CP2D which was isolated from Cipanas (Table 2).

Microorganisms growing under aerobic conditions need iron for a variety of functions, including reduction of oxygen for ATP synthesis, reduction of ribotide precursors of DNA, for formation of heme and for other essential purposes. A level of at least one micromolar iron is needed for optimum growth (Neilands 1995).

Table 2 Isolates of siderophore-producing rhizobacteria which produced inhibition zone against *R. solanacearum* on Kings's B agar

| Isolate code[1)] | Diameter of inhibition zone (mm) | Isolate code | Diameter of inhibition zone (mm) | Isolate code | Diameter of inhibition zone (mm) |
|---|---|---|---|---|---|
| **CP1B[2)]** | 6.8 | **CP2L** | 1.6 | LB1C | 1.6 |
| CP1C | 3.6 | **CP2S** | 2.6 | **LB1D** | 0.5 |
| CP2B | 2.3 | CP3E | 5.0 | **LB1E** | 0.5 |
| **CP2C** | 0.6 | **CP3M** | 2.2 | LB1L | 0.5 |
| CP2D | 7.0 | **CP3T** | 4.0 | | |
| **CP2H** | 4.5 | LB1A | 1.6 | | |

Note: 1) CP = isolates from Cipanas; LB = isolates from Lembang
2) Isolates written in bold were positively causing Hypersensitive Reaction (HR)

Table 3 Effect of siderophore-producing rhizobacteria on tomato seed viability of Arthaloka and Ratna varieties

| Treatment | Seed viability (%)*) |
|---|---|
| Var. Arthaloka | |
| Control | 93.33 ab |
| CP1C | 86.63 abc |
| CP2B | 88.83 abc |
| CP2D | 84.40 abc |
| CP3E | 88.87 abc |
| LB1A | 75.50 abc |
| LB1C | 100.00 a |
| LB1L | 95.53 a |
| Var. Ratna | |
| Control | 66.60 c |
| CP1C | 75.53 abc |
| CP2B | 79.97 abc |
| CP2D | 84.43 abc |
| CP3E | 75.53 abc |
| LB1A | 68.83 bc |
| LB1C | 66.63 c |
| LB1L | 84.30 abc |

Note: *) Means in the same column followed by the same letter are not significantly different according to Duncan Multiple Range Test ($p < 0.05$)

Table 4 Effect of siderophore-producing rhizobacteria on the increase of tomato plant height of Arthaloka and Ratna varieties

| Treatment | Plant height increase (cm)[1)] | | | | | | AUHPGC[3)] (cm days) |
|---|---|---|---|---|---|---|---|
| | I 5 DAP[2)] | II 10 DAP | III 15 DAP | IV 20 DAP | V 25 DAP | VI 30 DAP | |
| Var. Arthaloka | | | | | | | |
| Control | 1.04 abcde | 3.57 a | 2.03 b | 2.86 a | 4.34 a | 6.85 a | 83.75 a |
| CP1C | 0.99 bcde | 1.42 a | 3.83 a | 2.49 a | 4.53 a | 7.50 a | 82.56 a |
| CP2B | 1.51 abc | 2.30 a | 2.04 b | 2.65 a | 4.74 a | 7.20 a | 80.40 a |
| CP2D | 1.69 ab | 2.32 a | 2.24 b | 2.61 a | 4.66 a | 7.81 a | 82.90 a |
| CP3E | 1.23 abcd | 2.78 a | 2.00 b | 2.58 a | 3.93 a | 6.43 a | 75.53 a |
| LB1A | 1.66 ab | 2.48 a | 2.25 b | 3.05 a | 4.61 a | 6.98 a | 83.53 a |
| LB1C | 1.71 ab | 2.43 a | 1.81 b | 2.70 a | 4.44 a | 6.63 a | 77.78 a |
| LB1L | 1.87 a | 2.69 a | 1.81 b | 2.61 a | 4.19 a | 6.65 a | 77.78 a |
| Var. Ratna | | | | | | | |
| Control | 0.79 cde | 1.99 a | 1.89 b | 1.97 a | 3.19 a | 5.09 a | 59.90 a |
| CP1C | 1.09 abcde | 0.99 a | 3.73 a | 2.11 a | 3.98 a | 6.47 a | 72.92 a |
| CP2B | 0.29 e | 1.51 a | 1.89 b | 1.31 a | 2.85 a | 3.81 a | 48.05 a |
| CP2D | 0.48 de | 1.91 a | 2.03 b | 1.85 a | 2.97 a | 4.37 a | 56.00 a |
| CP3E | 0.87 bcde | 1.23 a | 1.87 b | 1.86 a | 2.89 a | 4.23 a | 52.00 a |
| LB1A | 0.91 bcde | 1.78 a | 1.46 b | 1.57 a | 2.29 a | 3.67 a | 46.97 a |
| LB1C | 0.53 de | 1.45 a | 2.0 b | 1.44 a | 2.57 a | 3.77 a | 48.17 a |
| LB1L | 0.40 de | 2.00 a | 1.81 b | 1.35 a | 2.27 a | 3.55 a | 47.09 a |

Note: 1) Means in the same column followed by the same letter are not significantly different according to Duncan Multiple Range Test ($p < 0.05$)
2) DAP = Days After Planting
3) AUHPGC = Area Under Height of Plant Growth Curve

Seven isolates of the bacteria were tested for their effects on seed viability, plant height and the fresh and dry weight of two varieties of tomato plants, i.e. Arthaloka and Ratna, as presented in Table 3, 4, and 5, respectively. Data in Table 3, 4, and 5 show not only that the isolates of bacteria did not significantly increase the seed viability, plant height, fresh and dry weight of tomato compared to control, but also they did not have harmful effects to the tomato plants. The characteristics of colony morphology, physiology and biochemistry aspects of the seven isolates were presented in Table 6 and 7, respectively.

Table 5 Effect of siderophore-producing rhizobacteria on fresh and dry weight of tomato plants

| Treatment[1)] | Fresh weight (g/plant)[2)] | Dry weight (g/plant) |
|---|---|---|
| Var. Arthaloka | | |
| Control | 15.439 a | 3.381 a |
| CP1C[1)] | 14.266 a | 2.587 a |
| CP2B | 15.070 a | 2.900 a |
| CP2D | 15.265 a | 2.986 a |
| CP3E | 9.515 a | 2.381 a |
| LB1A | 12.557 a | 2.721 a |
| LB1C | 12.572 a | 2.579 a |
| LB1L | 11.335 a | 2.742 a |
| Var. Ratna | | |
| Control | 10.109 a | 2.360 a |
| CP1C | 8.748 a | 1.954 a |
| CP2B | 6.887 a | 1.801 a |
| CP2D | 10.744 a | 2.281 a |
| CP3E | 9.566 a | 2.006 a |
| LB1A | 7.880 a | 1.547 a |
| LB1C | 6.887 a | 1.436 a |
| LB1L | 5.319 a | 1.535 a |

Note: 1) CP = isolates from Cipanas; LB = isolates from Lembang
2) Means in the same column followed by the same letter are not significantly different according to Duncan Multiple Range Test ($p < 0.05$)

Table 6 Morphological characteristic of colony of the seven isolates of siderophore-producing rhizobacteria on King's B Agar

| Isolate code[1)] | Colony characteristic | | | | |
|---|---|---|---|---|---|
| | Diameter | Color | Elevation | Edge | Form |
| CP1C | ± 1 mm | White | Convex | Wavy | Circular |
| CP2B | ± 1 mm | Broken white | Domed | Entire | Circular |
| CP2D | ± 1 mm | Broken white | Convex | Entire | Circular |
| CP3E | ± 1 mm | Dark yellow | Convex | Entire | Circular |
| LB1A | ± 1 mm | Greenish white | Convex | Entire | Circular |
| LB1C | ± 3 mm | Pale white | Umbonate | Curly | Irregular |
| LB1L | ± 1 mm | Broken white | Convex | Wavy | Circular |

Note: 1) CP = isolates from Cipanas; LB = isolates from Lembang

Table 7 Physiological and biochemistry characteristics of siderophore-producing rhizobacteria having potential as antagonist for *R. solanacearum*

| Isolate code[1)] | Fluorescence | Gram reaction | Phosphate solubilization | Resistance to 80 °C |
|---|---|---|---|---|
| CP1C | - | - | + | + |
| CP2B | +++ | - | + | - |
| CP2D | - | - | + | + |
| CP3E | - | - | - | - |
| LB1A | + | - | + | - |
| LB1C | - | + | + | + |
| LB1L | - | - | + | + |

Note: 1) CP = isolates from Cipanas; LB = isolates from Lembang

Based on the inhibition zone production, effect on seed viability, effect on plant height increase, and effect on fresh and dry weight of tomato product, two isolates of siderophore-producing bacteria having the best effects were selected. The two isolates were CP1C and CP2D. The sequence of 16S rDNA of those isolates were referred to the gene bank. Using the BLAST program the isolate of CP1C was identified as *Brevundimonas* sp., while the isolate CP2D was

Table 8 Maximum score, E value and percentage of similarities of siderophore-producing bacteria

| Isolate | Species homolog | Identity | Max score | Query cover | E value | Accession number |
|---|---|---|---|---|---|---|
| CP1C | *Brevundimonas* sp. 13630 G 16S ribosomal RNA gene, partial sequence | 95% | 2021 | 92% | 0.0 | EU741063.1 |
| CP2D | *Enterobacter* sp. enrichment culture clone DWSR 106 16S ribosomal RNA gene, partial sequence | 88% | 998 | 69% | 0.0 | JN944751.1 |

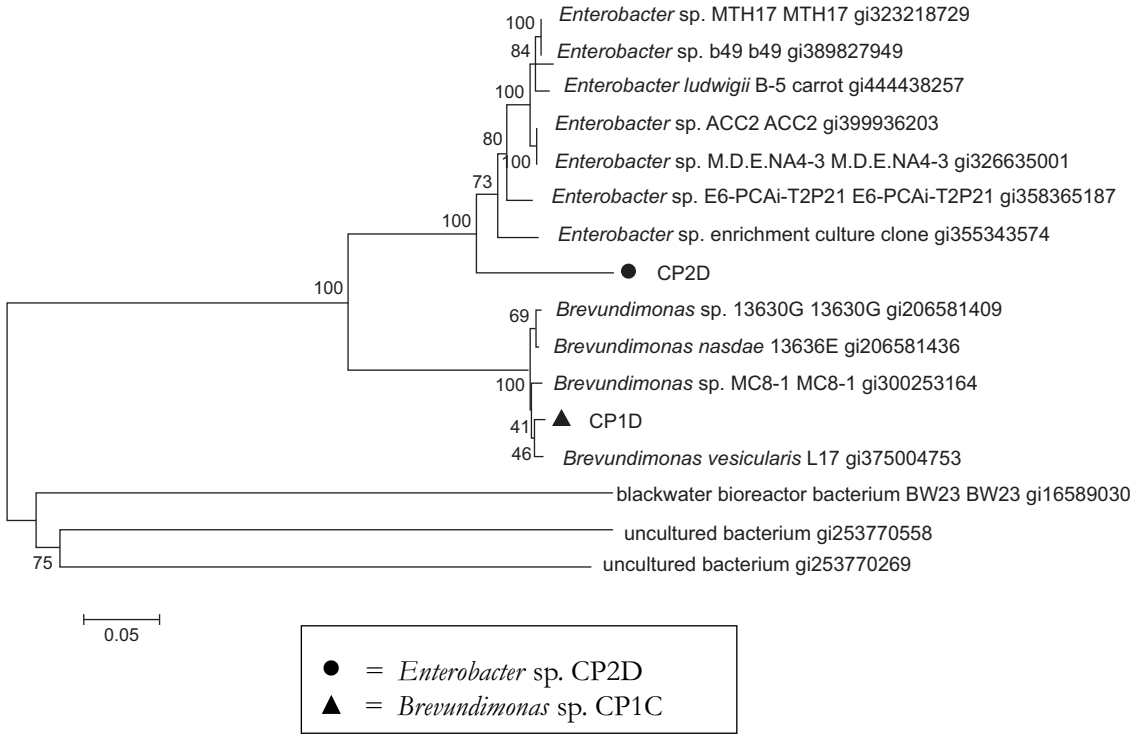


Figure 3 Phylogenetic tree of isolates *Enterobacter* sp. CP2D and *Brevundimonas* sp. CP1C

identified as *Enterobacter* sp. with percentage of similarity of 92% and 93%, respectively. Maximum score, E value and the percentage of similarities of siderophore-producing bacteria were presented in Table 8. Phylogenetic tree of the related bacteria is presented in Figure 3. Tian *et al.* (2009) reported that *Enterobacter* and *Pseudomonas* were dominant in the rhizosphere of tobacco, with 44.5% and 24.7% total frequency, respectively.

Siderophores are produced by various bacteria and fungi, usually classified by the ligands used to chelate the ferric iron. The major groups of siderophores include the catecholates (phenolates), hydroxamates and carboxylates (e.g. derivatives of citric acid) (Saharan & Nehra 2011). Rachid & Ahmed (2005) reported that streptomycin and penicillin added to succinate medium acts differently on the siderophores production. Streptomycin reduced siderophores production below 10 μM in different iron concentrations, while penicillin increased the production of siderophores in the presence of excess iron (above 100 μg/mL). The growth of *P. fluorescens* and siderophore production were inhibited by the occurrence of heavy metals (lead, mercury and cadmium), especially in iron-limited condition.

## CONCLUSIONS

The average of siderophore-producing bacteria isolated from Cipanas District (1.98 x $10^7$ cfu/g) was not significantly different from those isolated from Lembang District (5.3 x $10^7$ cfu/g). The highest diameter of inhibition zone to *Ralstonia solanacearum* was 7.0 mm, produced by isolate CP2D. The selected bacteria producing the inhibition zone did not significantly affect seed viability, plant growth, fresh weight and dry weight of tomato compared to control. Based on the characteristics of colony morphology, physiology, biochemistry and partial sequence of 16S rDNA, two selected isolates, i.e. CP1C and CP2D were identified as *Brevundimonas* sp. and *Enterobacter* sp., respectively.

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