Biological control of *Sclerotium rolfsii* on tomato seedlings using *Bacillus* spp. consortium

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Abstract. The *Sclerotium rolfsii* which widely distributed soil-borne plant pathogen, causes many economically important crops severely reduce their yield at worldwide extend, including tomato and are very difficult to control due to its sclerotia. The beneficial plant-microbe interactions play crucial roles in protection against large number of plant pathogens causing disease. Our previous research had screened 9 *Bacillus* spp. strains which had ability to control *R. syzigii* subsp. *indonesiensis* and promote growth and yield of solanaceous plants. Those strains were *Bacillus cereus* AGBE 1.2 TL, *Bacillus toyonensis* AGB E 2.1 TL, *Bacillus cereus* SLB E 1.1 SN, *Bacillus cereus* SLB E 1.1 AP, *Bacillus pseudomycooides* SLB E 3.1 AP, *Bacillus cereus* SLB E 1.1 BB, *Bacillus cereus* SLB E 2.1 BB, *Bacillus thuringiensis* SLB E 2.3 BB, and *Bacillus cereus* SLB E 3.1 BB. Our current study objective was to develop the best consortium to control *S. rolfsii* and promote growth of *S. rolfsii* seedlings. The research designed in experimental methods and was arranged in a completely randomized design with 7 treatments and triplications. The methods done were compatibility assay of the *Bacillus* strains, development of consortium based from the compatibility, and consortium assay to control *S. rolfsii* and promote growth of tomato seedlings. The result showed, out of the 6 compatible consortiums, consortium A4 consists of *Bacillus* strain AGBE 1.2 TL, SLBE 3.1 AP, SLBE 2.1 BB and SLBE 3.1 BB were shown to be the best to control *S. rolfsii* on tomato seedlings with 85.00% of the seedlings were shown no symptoms. The consortium also had the best ability to promote growth of tomato seedlings up to 25.00% compared with control.

1. Introduction

Currently, few effective fungicides are available to control these harmful pathogens and, when allowed, chemical tools involve technical [e.g., onset of fungicide resistance in pathogen isolates], environmental [e.g., contamination of underground waters] and toxicological risks [e.g., chemical residues in vegetable products and derived foods][1].

Biological control is one of a desirable approach because control with other methods gives variable results [2] and since biological control is a key component of integrated disease management, it is important to search for biological control as promising alternatives to replace chemical pesticide and fertilizer in sustainable and organic agricultural systems [3].

The consortium are microorganisms from different species that act together in community. In a consortium, the organisms work together in a complex and synergistic way [4]. Evaluation of the compatibility and synergism of microbial components is essential in the ability of a microorganism consortium as biocontrol agents and biofertilizers [5]. Another study had used a consortium of 3 types of selected rhizobacteria to control the wilting of tomato plants by *Sclerotina sclerotiorum* and to promote the growth of tomato plants[6]. The consortium consist of PGPR strains treatment had ability...
to promote the plant growth and enhanced the production of ginger rhizome by 45.8%. In field experiments, the PGPR strain consortium could also reduce yellow and rhizome rot incidences on ginger by about 50.5%, which was comparable to that of a carbendazim and mancozeb fungicide mixture[7].

The current study was done to develop the best consortium to control S. rolfsii and promote the growth of tomato seedlings.

2. Methods
The research designed in experimental methods and was arranged in a completely randomized design with 7 treatments and triplications.

2.1. Rejuvenation and confirmation of PGPR and cyanobacteria isolates
The strains were obtained from Yanti’s collection [Unpublished], rejuvenated by the scratch method on Nutrient Agar [NA] medium and incubated for 2x24 hours.

| Code | Strain |
|------|--------|
| B1   | Bacillus cereus AGBE1.2TL, Bacillus toyonensis AGBE2.1TL, Bacillus cereus SLBE1.1SN, Bacillus cereus SLBE1.1AP, Bacillus pseudomycoides SLBE3.1AP, Bacillus cereus SLBE2.1BB, Bacillus cereus SLBE2.1BB |
| B2   | Bacillus cereus SLBE1.1SN, Bacillus cereus SLBE1.1AP, Bacillus pseudomycoides SLBE3.1AP, Bacillus cereus SLBE3.1BB |
| B3   | Bacillus cereus SLBE1.1BB, Bacillus cereus SLBE2.1BB, Bacillus thuringiensis SLBE2.3BB, Bacillus cereus SLBE3.1BB |

2.2. Compatibility test of PGPR and Cyanobacteria isolates
Compatibility between each PGPR and cyanobacteria isolates was tested by inhibition zones appeared by crossing the endophytic bacteria on NA medium in a petri dish. The shown halo zones on the cross-culture of the bacteria shown incompatibility.

2.3. Test of the ability of the consortium to control S. rolfsii on tomato
The consortium was prepared by combining 2-3 compatible species [2.2]. 1 mL of culture suspension was put into 24 mL of sterile coconut water and incubated for 2x24 hours. The consortium designed were shown in table 2.

| Code | Strain |
|------|--------|
| A1   | Bacillus toyonensis AGBE2.1TL, Bacillus cereus SLBE1.1AP, Bacillus pseudomycoides SLBE3.1AP, Bacillus cereus SLBE2.1BB, Bacillus cereus SLBE1.1AP, Bacillus pseudomycoides SLBE3.1AP, Bacillus cereus SLBE2.1BB, Bacillus thuringiensis SLBE2.3BB |
| A2   | Bacillus cereus SLBE1.1SN, Bacillus cereus SLBE1.1AP, Bacillus pseudomycoides SLBE3.1AP, Bacillus cereus SLBE2.1BB, Bacillus thuringiensis SLBE2.3BB |
| A3   | Bacillus cereus SLBE1.1BB, Bacillus cereus SLBE2.1BB, Bacillus thuringiensis SLBE2.3BB, and Bacillus cereus SLBE3.1BB |
| A4   | Bacillus strain AGBE1.2TL, SLBE3.1AP, SLBE2.1BB, and SLBE3.1BB |
A5 Bacillus cereus AGBE1.2TL, Bacillus cereus SLBE1.1AP, Bacillus pseudomycoides SLBE3.1AP, Bacillus cereus SLBE1.1BB, Bacillus cereus SLBE2.1BB, Bacillus thuringiensis SLBE2.3BB, and Bacillus cereus SLBE3.1BB.

A6 Bacillus cereus AGBE1.2TL, Bacillus toyonensis AGBE2.1TL, Bacillus cereus SLBE1.1SN, Bacillus cereus SLBE1.1AP, Bacillus pseudomycoides SLBE3.1AP,

2.3.1. The introduction Bacillus consortium. The consortium was introduced into the seeds and tomato seeds two times each for 15 minutes before sowed in the seed tray and before planting the seedlings.

2.3.2. Pathogens Innoculation. S. rolfsii was acquired from diseased plants, isolated and cultured on Potato Dextrose agar. S. rolfsii inoculated one week before planting with 10 gr of pathogen cultured in rice [1 week old].

3. Results and Discussions
PGPR lives in association with roots and stimulates the plant growth and/or reduces the incidence of plant disease[8]. The consortiums developed in this study had showed to increase height and total of leaves on tomato plants treated with consortiums. The increased growth had improved results when compared with the previous study which uses one strain for treatments. This effect occurrence may due to the ability of the Bacillus strains to increase the growth of tomato synergically.

Table 3. Bacillus strains Compatibility Assay, √= no inhibition zone appear; - = incompatible

| Strain | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 |
|--------|----|----|----|----|----|----|----|----|----|
| B1     | -  |    |    |    |    |    |    |    |    |
| B2     |    |    |    |    |    |    |    |    |    |
| B3     | √  |    |    |    |    |    |    |    |    |
| B4     |    | √  |    |    |    |    |    |    |    |
| B5     | √  | √  | √  | √  |    |    |    |    |    |
| B6     |    |    |    |    |    | √  |    |    |    |
| B7     | √  | √  | √  | √  | √  | -  |    |    |    |
| B8     | √  |    | √  | √  | -  | -  |    |    |    |
| B9     | √  |    |    | √  | √  | √  | √  | √  |    |

Our current study showed that the tomato plants treated with consortiums shown earlier flowering time and yields increased. The beneficiary effect could be due to the promoted growth by consortiums on tomato which later could also lead to accelerated flowering time and yields increase. The yields increasement response also showed better when threated in consortiums compared to single strain inoculants.

Table 4. Growth Promoting activity of the Bacillus consortium on tomato
### Table 5. Consortium ability as biocontrol agents of *S. rolfsii* on tomato

| consortia | Seed Germination [%] | Effectivity [%] | Seedlings’ height [cm] | Effectivity [%] | No. of leaves | Effectivity [%] |
|-----------|----------------------|-----------------|------------------------|-----------------|--------------|----------------|
| A4        | 96.67                | 31.81           | 9.73 a                 | 24.74           | 4.00 a       | 11.11          |
| A3        | 93.33                | 27.26           | 9.72 a                 | 24.61           | 4.00 a       | 11.11          |
| A5        | 90.00                | 22.72           | 9.12 abc               | 16.92           | 4.00 a       | 11.11          |
| A6        | 90.00                | 22.72           | 8.90 abc               | 14.10           | 4.00 a       | 11.11          |
| A2        | 86.67                | 28.17           | 8.75 abc               | 12.18           | 4.00 a       | 11.11          |
| A1        | 86.67                | 18.17           | 7.94 abcd              | 1.79            | 3.60 ab      | 0.00           |
| Control   | 73.34                | -               | 7.80 abcd              | -               | 3.60 ab      | -              |

Note: Means with the same letter are not significantly different by LSD test at p < 0.05

The treatment consists of Bacillus strains consortium consistently suppressed the *S. rolfsii* disease on this current study. The disease emergence and severity are shown lowered compared to untreated control [Table 5] and 2 consortium’s treated tomato had no visible symptom observed, the A4 and A3 until the last day of observations. The results had suggested that the compatible *Bacillus* strain in the consortia had ability to suppress the *S. rolfsii* disease. The consortium of *Bacillus* spp. strains as biocontrols were one of promising approach as disease and pest control technique. Moreover, the synergetic beneficiary effects of the consortiums from combined biocontrol agents may enhance the induced systemic resistance. These effects occurred because the consortium synergetic effect could increase the reliability, efficacy, and consistency in more type of soil and environmental[9].

### Table 5. Consortium ability as biocontrol agents of *S. rolfsii* on tomato

| consortium | Pre-emergence [%] | Effectivity [%] | Post-emergence [%] | Incubation period[dai] | Effectivity [%] |
|------------|------------------|-----------------|---------------------|------------------------|----------------|
| Control    | 31.46            | 55.56           | 14.40               | e                      |
| A2         | 17.36            | 44.82           | 9.3                 | 25.00 d                | 73.61          |
| A1         | 15.16            | 51.88           | 8.89                | 25.00 d                | 73.61          |
| A5         | 13.20            | 58.04           | 8.89                | 26.80 b                | 86.11          |
| A6         | 13.10            | 58.36           | 4.55                | 25.60 c                | 77.78          |
| A3         | 11.12            | 64.65           | 55.56               | 30.00* a               | 108.33         |
| A4         | 9.06             | 71.20           | 21.88               | 30.00* a               | 108.33         |

4. Conclusion
In conclusion, treatment of Bacillus consortium on tomato was shown beneficial effect in *S. rolfsii* control. Out of the *Bacillus* spp. strains combination from the 6 compatible consortiums, the consortium A4 consists of *Bacillus* strain AGBE 1.2 TL, SLBE 3.1 AP, SLBE 2.1 BB, and SLBE 3.1 BB were shown to be the best to control *S. rolfsii* on tomato seedlings were shown no symptoms. This
current research has shown that biocontrol agents combination as consortiums resulted in reduction of *S. rolfsii* disease emergence and development compared with control. The A4 consortium has also shown 31.81% higher germination rate, 24.74% higher seedling’s height and 11.11% more leaves on treated tomato leaves compared with control.

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