In planta screening of chili roots’ endophyte bacteria to control bacterial wilt disease

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Abstract. Bacterial wilt is an important disease of chili. This disease cause by Ralstonia syzygii subsp. indonesiensis. This pathogen causing plant death and up to 100% yield loss. The economical impact due to this pathogen problem, even though this pathogen had been controlled. Bacterial Endophyte usage which are one of biological control strategy were one of the considered alternative method which are also compatible with sustainable agriculture program. This study objected to obtain the endophyte bacteria strains from healthy roots of chili that had ability to control bacterial wilt and promote growth and yields. Bacterial endophytes were isolated from the roots of healthy chili plants at the bacterial wilt disease endemic area in West Sumatera Province, Indonesia. The screening methods used in this study were in planta technique which the approach was focused on indirect mechanism (induced systemic resistance). Among the 14 of 16 endophyte bacteria strains were found to had ability to control bacterial wilt on chili without any symptoms appear (100%). We have found three bacterial endophyte strains (SLBE.3.1.BB7, SLBE.3.1.AP3 and AGBE4.1.TL5, which have multiple traits as biocontrol of bacterial wilt disease and as growth promoter and yields of chili.

Keywords: chili, endophytic bacteria, healthy root, in planta technique, Ralstonia syzygii subsp. indonesiensis.

1. Introduction

Bacterial wilt cause by a soil-borne vascular pathogen, Ralstonia syzygii subsp. indonesiensis Safni et al. (2014) (formerly Ralstonia solanacearum Yabuuchi et al. 1995) is very common in the field, such as chili (Capsicum annum L.). Once this pathogen is existed in a field it is difficult to control [1]. Bacterial wilt causing the yield loss of many crops such as tomato, chili, eggplant, tobacco, and potato [2].

Bacterial wilt disease control is difficult because of the pathogen high variability, limited options of chemical pesticide, high survival rate of the pathogen in various environments and its wide host range. Biological control is desirable because control with other methods gives variable results [3]. As one of the key components of Integrated disease management, endophyte bacteria as biological control options is an important to study because its promising alternative to replace chemical pesticide and fertilizer in sustainable and organic agricultural systems [4]. In the last decades, bacterial endophyte
have attracted more and more concerns as novel resource in biocontrol of plant diseases and promotion of plant growth [5].

Bacterial endophytes are well known to colonize in plant tissue, in the cell walls’ intercellular space and roots’ xylem, stem and leaves, and also found in flower tissues[7], fruits and seeds[8]. As a rule more endophytes are found in the roots of plants than other plant parts [9]. The relatively steady internal environment inside the plant tissues makes bacterial endophytes more bioactive than the rhizospheric or others plant associated microorganisms. Use of indigenous bacterial endophyte is considered as an environmentally-friendly an ecologically efficient strategy [10].

Growth promoting ability by bacterial endophytes could be directly established by phytohormones productions, induced endogenous phytohormone production due to their interactions with plants or increases accessibilities of plant nutrients such as phosphor and nitrogen [11]. The biocontrol ability of the associated bacterial endophyte to increased resistance properties were by the ability to produce various compounds, such as antibiotics or enzyme such as chitinase, which inhibit the pathogens growth in the plans, and thus act as biocontrol agents [11-15]. Bacterial endophytes were also found to have the ability to stimulate defense mechanism that termed as induced systemic resistance (ISR) which provided a next level of protection of plants to wider spectrum of pathogens [16] such as nematode, fungi, bacteria and virus.

Some isolates of bacterial endophyte showed antagonistic effect on Globodera rostochiensis in vitro and in vivo [17]. Bacterial endophyte originated from roots of Cyperus rotundus are able to reduce the number of Meloidogyne incognita in the roots of plants and are able to able to reduce the number of gall on the roots of tomato plants infested with M. incognita [18]. Five bacterial endophyte isolates from different parts ant at different growth stages of soybean are considered as efficient to control some fungal pathogens on soybean. They havee dual abilities i.e. antagonistic and plant growth promotion with the view of plant health and yield [10]. Bacterial endophyte could control angular leaf spot cause by Xanthomonas axonopodis pv. malvacearum on cotton [19]. Curtobacterium flaccumfaciens as endophyte in citrus plant could increase the resistance of citrus against Xylella fastidiosa [20] and reduced the severity of the disease symptoms induced by X. fastidiosa in Catharanthus roseus [21]. Bacteria endophyte from potatoes could inhibit growth of Erwinia and Xanthomonas [22]. Two bacterial endophyte isolates from healthy soybean root showed as the best to control bacterial pustule on soybean cause by X. axonopodis pv. glycines [23]. Six bacterial endophyte isolates from onion roots showed better performance in induced the resistance of onion against X. axonopodis pv. allii and increased the yield of onion [24]. Three bacterial endophyte from different parts of chili showed the growth promoting activity, reduction of disease incidence of five genus of fungal pathogens and high yield under field condition. These isolates showing multi attributes that can significantly influence of chili growth [25]. The information about bacterial endophyte to control bacterial wilt disease on chili are still limited.

The objective of these experiment was to obtain the bacterial endophyte strains from healthy chili roots, which have multi functions as biocontrol against bacterial wilt and biofertilizer to increase growth and yield of chili.

2. Materials and Methods

2.1. Isolation of bacterial endophyte from healthy chili’s roots.

Chili’s root samples were collected from healthy chili in endemic area of bacterial wilt in Taluak Village, Banuhampu District, Agam Region and Alahan Panjang Village, Lembah Gumanti District, Solok Region, West Sumatera, Indonesia. Bacterial wilt disease on chili was collected also at the same location. Samples were stored in a refrigerator (at 5°C) Sample were tagged and brought to the laboratory. The procedures of isolation of bacterial endophyte were used according to Misagi and Donndelinger (1990) [26]. Plant samples were disinfected and subsequently crushed with a laboratory. Aliquots of 100 μl of the resulting plant juices was placed in a Petri dish to which 10 ml of Nutrient Agar (NA) were added and s27] stirred well. Petri dish placed at room temperature for 2 days (about 30°C). Thereafter, the dominant colonies suspected as biocontrol agents were further purified on NA
and their single colony were transferred to microtube contain 1mL of sterilized water as stock culture and stored in refrigerator.

2.1.1. *Isolation of R. syzygii subsp. indonesiensis*. *R. syzygii subsp. indonesiensis* was isolated from bacterial wilt diseased plant parts using Tetrazolium chloride (TZC) medium and incubated at 30 °C for 48 h. Pathogenicity of the pathogens were assayed by method of Winstead and Kelman [27]. And the most virulence strains were used for further assay.

2.2. *In planta screening of selected bacterial endophyte strains on seedlings’ stage.*

The experiments were conducted in a greenhouse to evaluate the ability of bacterial endophyte to promote growth of chili’s seedlings. The experiment was designed as randomized complete. It was consisted of 17 treatments and 6 replicates. The treatments were 16 bacterial endophyte strains and control.

2.2.1. *Multiplication of endophytic bacteria.* The endophyte bacteria from stock culture were grown on NA for 72h, then the inoculum were transferred to 100mL of Nutrient Broth (NB) in 250 mL Erlenmeyer flask as pre-culture, and cultured on a shaker at 150 rpm, room temperature 48h. The main-culture were produced by transferring 1 mL of preculture suspension to 50 mL of sterilized coconut water in a flask, incubated the same as preculture condition, then the density were determined comparing with scale 8 of McFarland solutions (approximately 10⁸CFU/mL)[28].

2.2.2. *Inoculation of bacterial endophyte.* Seeds used in this study were obtained from the native chili of Taluak Village, Banuhampu Districh, Agam Residence, West Sumatra which selected by the best performance. Germination rate test were done by common methods using paper [29]. The bacterial endophyte main-culture stock were applied as seed inoculation, soaked for 15 minutes and shade-dried for 30 minutes then sowed with 2 seeds/pot at pot tray. Seedings and planting were all use the agronomical practices recommend for chili in Indonesia. Growth obserations were done on the 5 random-selected seedlings for 15 days after sowing. Parameter observed (height and total of leaves) were observed at 21 days old seedlings.

2.3. *In planta evaluation of bacterial endophyte strains to control of bacterial wilt disease on chili*

The experiment were done in screen house using completely randomized design, consisted of 18 treatments with 6 replications. The treatments were 16 bacterial endophyte strains, antibiotic and control. The bacterial endophyte strains were inoculated on three weeks old chili seedlings by root dipping technique before transplanting. Suspension of *R. syzygii subsp. indonesiensis* (10⁸ cells/mL) were inoculated on 1 months old chili by stem injection. Disease assesments were observed as follow: incubation period, disease incidence and disease severity. To examine the effect of bacterial endophyte on the plant growth characteristics were counted plant height, number of leaves, generative phase, and fruit weight.

3. *Results and discussion*

3.1 *Isolation of potential bacterial endophyte as biocontrol agents*

Sixteen bacterial endophyte strains were isolated from healthy chili’s roots. The diversity of bacterial endophyte from healthy root of chili varied with regard to morphology of colony and Gram. The colony characters of the bacterial endophyte strains showed the diverse colony shapes, diameter, colors, margins, elevation. They were consisted of 10 colony types (Table 1). Healthy chili roots were dominated by Gram positive (13 strains), white color (11 strains), irregular shape (9 strains). All of bacterial endophyte strains showed negative for the hypersensitive reaction (HR). It is mean they are nonpathogenic, and they were tested for the next experiment.
whereas control plant the disease incidence 80 % introduced with 14 bacterial experiment post inoculation, dpi) or without wilt symptom compared with control (22.2 dpi) until the end of experiment. The disease incidence varied between 0-20 %, especially on plants, which were introduced with 14 bacterial endophyte strains and streptomycin sulphate treatment become 0 %, whereas control plant the disease incidence 80 % (Figure 3).

### 3.2 In planta screening of selected bacterial endophyte strains to increase growth of chili seedlings
All indigenous bacterial endophyte strains could enhance significantly germination of chili seeds (83.3-100.0 %) and the rate of enhancement varied 19.00-42.85 % over non treated control (70 %) (Table 2 and Figure 2). Seedling’s height also was increased after inoculation of indigenous bacterial endophyte strains (7.14-9.21 cm) and the enhancement varied 25.04-53.75 % compare to control (5.99 cm).

Seed inoculation with all indigenous bacterial endophyte isolates significantly enhanced seed germination from 83.3-100.0 % and the rate of enhancement varied 19.00-42.85 % over non treated control (70 %) (Table 2 and Figure 1). Seedling’s height also increased after inoculations varied from 7.14-9.21 cm and the enhancement varied 25.04-53.75 % compare to control (5.99 cm). Number of leaves of indigenous bacterial endophyte introduced chili seedlings varied from 3.40-4.00 pieces and the enhancement 13.33-33.33 % compare to control (3.00 pieces) The best isolates to promote growth of chili seedlings were SLBE.3.1.BB7, SLBE.1.2.BB5, AGBE.3.1.TL9, SLBE.2.2.AP6, SLBE.1.1.BB4 and AGBE.2.1.TL10.

### 3.3. In planta screening of selected endophytic bacterial strains to control bacterial wilt and to increase growth and yield of chili.
Under greenhouse conditions, results clearly confirm that plant treated bacterial endophyte strains significance reduced disease compare to infected control (Table 3). *R. syzigii* subsp. *indonesiensis*-incubation period in chili treated with bacterial endophyte strains were shown longer (39.0-46.0 days post inoculation, dpi) or without wilt symptom compared with control (22.2 dpi) until the end of experiment. The disease incidence varied between 0-20 %, especially on plants, which were introduced with 14 bacterial endophyte strains and streptomycin sulphate treatment become 0 %, whereas control plant the disease incidence 80 % (Figure 3).

### Table 1. The characters of bacterial endophyte strains from healthy chili’s root

| Nr. | Bacterial endophyte isolate | Type   | Shape     | Elevation | Margin | Diameter (mm) | Colour | Gram reaction | Hyper-sensitive Reaction |
|-----|-----------------------------|--------|-----------|-----------|--------|---------------|--------|---------------|-------------------------|
| 1   | SLBE.1.1.AP1                | 1      | Irregular | Raised    | Lobate | 0.2           | Red    | -             | -                       |
| 2   | SLBE.1.1.SN1                | 1      | Irregular | Raised    | Lobate | 0.7           | Red    | -             | -                       |
| 3   | SLBE.2.1.BB2                | 2      | Irregular | Raised    | Lobate | 0.5           | White  | +             | -                       |
| 4   | SLBE.3.1.AP3                | 3      | Irregular | Raised    | Wavy   | 0.4           | White  | +             | -                       |
| 5   | SLBE.1.1.BB4                | 4      | Irregular | Flat      | Wavy   | 0.3           | White  | +             | -                       |
| 6   | SLBE.2.3.BB4                | 4      | Irregular | Flat      | Wavy   | 0.6           | White  | +             | -                       |
| 7   | SLBE.1.2.BB5                | 5      | Circular  | Raised    | Entire | 0.2           | White  | +             | -                       |
| 8   | AGBE.1.1.TL5                | 5      | Circular  | Raised    | Entire | 0.3           | White  | +             | -                       |
| 9   | AGBE.4.1.TL5                | 5      | Circular  | Raised    | Entire | 0.4           | White  | +             | -                       |
| 10  | SLBE.2.2.AP6                | 6      | Rhizoid   | Flat      | Rhizoid| 2.2           | White  | +             | -                       |
| 11  | SLBE.3.3.AP6                | 6      | Rhizoid   | Flat      | Rhizoid| 1.0           | White  | +             | -                       |
| 12  | SLBE.3.1.BB7                | 7      | Irregular | Flat      | Lobate | 1.5           | White  | +             | -                       |
| 13  | SLBE.4.2.BB8                | 8      | Irregular | Umbonate  | Lobate | 0.3           | White  | +             | -                       |
| 14  | AGBE.1.2.TL9                | 9      | Circular  | Raised    | Entire | 0.2           | Yellow | +             | -                       |
| 15  | AGBE.3.1.TL9                | 9      | Circular  | Raised    | Entire | 0.2           | Yellow | -             | -                       |
| 16  | AGBE.2.1.TL10               | 10     | Irregular | Flat      | Wavy   | 0.6           | Transparent | +            | -                       |
Table 2. Germination rate and seedling’s growth of bacterial endophyte introduced chili (21 days post inoculation, dpi)

| Bacterial endophyte strains | Germination rate (%) | Enhanced (%) | Seedling’s height (Cm) | Enhanced (%) | Number of leaves (Pieces) | Enhanced (%) |
|-----------------------------|----------------------|--------------|------------------------|--------------|--------------------------|--------------|
| SLBE.1.1.AP1                | 93.3                 | 34.14        | 8.12                   | abc          | 35.55                    | 4.00a        | 33.33        |
| SLBE.1.1.SN1                | 96.7                 | 38.14        | 8.01                   | abc          | 33.72                    | 4.00a        | 33.33        |
| SLBE.2.1.BB2                | 100.0                | 42.85        | 8.42                   | abc          | 40.56                    | 4.00a        | 33.33        |
| SLBE.3.1.AP3                | 90.0                 | 28.57        | 8.29                   | abc          | 38.39                    | 4.00a        | 33.33        |
| SLBE.1.1.BB4                | 96.7                 | 38.14        | 8.95                   | ab           | 49.41                    | 4.00a        | 33.33        |
| SLBE.2.3.BB4                | 83.3                 | 19.00        | 8.21                   | abc          | 37.06                    | 3.90a        | 30.00        |
| SLBE.1.2.BB5                | 93.3                 | 34.14        | 9.17                   | ab           | 53.08                    | 4.00a        | 33.33        |
| AGBE.1.1.TL5                | 93.3                 | 34.14        | 8.51                   | abc          | 42.07                    | 3.60ab       | 26.66        |
| AGBE.4.1.TL5                | 90.0                 | 28.57        | 8.11                   | abc          | 35.39                    | 4.00a        | 33.33        |
| SLBE.2.2.AP6                | 100.0                | 42.85        | 9.13                   | ab           | 52.42                    | 4.00a        | 33.33        |
| SLBE.3.3.AP6                | 96.7                 | 38.14        | 8.39                   | abc          | 40.06                    | 4.00a        | 33.33        |
| SLBE.3.1.BB7                | 90.0                 | 28.57        | 9.21                   | a            | 53.75                    | 4.00a        | 33.33        |
| SLBE.4.2.BB8                | 96.7                 | 38.14        | 7.49                   | c            | 25.04                    | 3.40bc       | 13.33        |
| AGBE.1.2.TL9                | 96.7                 | 38.14        | 8.44                   | abc          | 40.90                    | 3.60ab       | 26.66        |
| AGBE.3.1.TL9                | 100.0                | 42.85        | 9.17                   | d            | 53.08                    | 4.00a        | 33.33        |
| AGBE.2.1.TL10               | 100.0                | 42.85        | 8.92                   | ab           | 48.91                    | 4.00a        | 33.33        |
| control                     | 70.0                 | 0.00         | 5.99                   | d            | 0.00                     | 3.00c        | 0.00         |

Means with the same letter are not significantly different by least significant difference at p < 0.05.

**Figure 2.** Growth of indigenous endophytic bacterial inoculated chili seedlings compare to control (21 days after seeding). A. SLBE.1.1.BB; B. SLBE.2.2.BB; C. SLBE.3.1.BB; D. AGBE.3.1.TL; E. SLBE.4.2.BB and F. control
Table 3. Disease incidence of bacterial wilt disease on bacterial endophyte introduced chili

| Nr. | Bacterial endophyte strain | Incubation Period | Disease Incidence |
|-----|---------------------------|-------------------|-------------------|
|     |                           | Days | Prolonged (%) | % | Reduced % |
| 1.  | SLBE.1.1.AP1              | 46.0a* | 100.00 | 0 | 100 |
| 2.  | SLBE.1.1.SN               | 46.0a* | 100.00 | 0 | 100 |
| 3.  | SLBE.2.1.BB               | 46.0a* | 100.00 | 0 | 100 |
| 4.  | SLBE.3.1.AP3              | 46.0a* | 100.00 | 0 | 100 |
| 5.  | SLBE.1.1.BB               | 46.0a* | 100.00 | 0 | 100 |
| 6.  | SLBE.2.3.BB               | 46.0a* | 100.00 | 0 | 100 |
| 7.  | SLBE.1.2.BB               | 46.0a* | 100.00 | 0 | 100 |
| 8.  | AGBE.1.1.TL5              | 41.2a | 85.58 | 20 | 75 |
| 9.  | AGBE.4.1.TL5              | 46.0a* | 100.00 | 0 | 100 |
| 10. | SLBE.2.2.AP6              | 39.0a  | 75.67 | 20 | 75 |
| 11. | SLBE.3.3.AP6              | 46.0a* | 100.00 | 0 | 100 |
| 12. | SLBE.3.1.BB               | 46.0a* | 100.00 | 0 | 100 |
| 13. | SLBE.4.2.BB               | 46.0a* | 100.00 | 0 | 100 |
| 14. | AGBE.1.2.TL9              | 46.0a* | 100.00 | 0 | 100 |
| 15. | AGBE.3.1.TL9              | 46.0a* | 100.00 | 0 | 100 |
| 16. | AGBE.2.1.TL10             | 46.0a* | 100.00 | 0 | 100 |
| 17. | Streptomycine sulphate    | 46.0a* | 100.00 | 0 | 100 |
| 18. | Control                   | 22.2b | 0 | 80 | 0 |

Means with the same letter are not significantly different by least significant difference at p < 0.05.

Note: *The plants were still live until the end of experiment.

Not all bacterial endophyte strains could enhance the growth of chili compare than control plant. The plant height was increased on fourteen bacterial endophyte strains inoculated plants (56.68-84.98 cm) and the enhancement varied between 0.39-50.51 % compare than control plant (56.46 cm) (Table 4 and Figure 3). The number of leaves were increased on thirteen bacterial endophyte strains inoculated plants (210-270 pieces), the enhancement varied between 6.82-37.34 % compare than control (196.60 pieces). Whereas three bacterial endophyte strains and streptomycine sulphate treated plants showed a lower leaves number (149.0-194.6 pieces). The best bacterial endophyte strains to promote plant growth were SLBE.4.2BB8, SLBE.3.3.AP6 and AGBE.4.1.TL5.

The generative phase was earlier on three bacterial endophyte strains inoculated chili (48.00-49.20 days after transplanting, DAT) compare to control (56.6 DAT) (Table 5). Fruit yield increased on ten bacterial endophyte strains inoculated chili (44.83-83.80 g/plant), the enhancement 9.57-92.25 % compare to control (43.59 g/plant). The best strains to increase the fruit yield were SLBE.3.1.BB7, SLBE.3.1.AP3 and AGBE.4.1.TL5.

The diversity of bacterial endophyte in healthy chili roots were varied based on morphological characters of colonies and Gram reaction, they were consisted of 10 colony types. Bacteria endophytes were dominated by Gram positive. Similar results had reported by Amaresan et al., (2014) the
dominated species from chili roots were Bacillus sp. or Gram positive [25]. Bacterial endophyte community are diverse and extent of diversity may vary significantly between plant species.

![Image of bacterial endophytic introduced chili after inoculation](A) and wilted control plant (B).

**Figure 2.** Performance of bacterial endophytic introduced chili after R. syzygii subsp. indonesiensis inoculation (21 dpi) (A). Wilted control plant (B).

**Table 4.** Effect of bacterial endophyte isolates on growth of chili in pot experiment

| Nr. | Bacterial endophyte isolate | Plant height cm | Enhance (%) | Number of leaves | Enhance (%) |
|-----|-----------------------------|-----------------|-------------|-----------------|-------------|
| 1.  | SLBE.1.1.AP1                | 56.04           | -0.74       | 217.60          | 10.68       |
| 2.  | SLBE.1.1.SN1                | 57.80           | 2.37        | 261.00          | 32.76       |
| 3.  | SLBE.2.1.BB2                | 63.64 ab        | 12.72       | 238.20 ab       | 21.16       |
| 4.  | SLBE.3.1.AP3                | 58.50 bc        | 3.61        | 216.40 ab       | 10.07       |
| 5.  | SLBE.1.1.BB4                | 61.78 b         | 9.42        | 249.20 ab       | 26.76       |
| 6.  | SLBE.2.3.BB4                | 64.86 ab        | 14.88       | 210.00 abc      | 6.82        |
| 7.  | SLBE.1.2.BB5                | 62.10 ab        | 9.99        | 194.60 abc      | -1.02       |
| 8.  | AGBE.1.1.TL5                | 37.70 c         | -33.23      | 149.00 c        | -24.21      |
| 9.  | AGBE.4.1.TL5                | 70.68 ab        | 25.19       | 270.00 a        | 37.34       |
| 10. | SLBE.2.2.AP6                | 59.06 bc        | 4.61        | 178.80 bc       | -9.05       |
| 11. | SLBE.3.3.AP6                | 71.70 ab        | 26.99       | 249.00 ab       | 26.65       |
| 12. | SLBE.3.1.BB7                | 62.76 bc        | 11.16       | 231.40 ab       | 17.70       |
| 13. | SLBE.4.2.BB8                | 84.98a          | 50.51       | 238.40 ab       | 21.26       |
| 14. | AGBE.1.2.TL9                | 56.68 bc        | 0.39        | 254.80 ab       | 29.60       |
| 15. | AGBE.3.1.TL9                | 62.64 bc        | 10.95       | 244.20 ab       | 24.21       |
| 16. | AGBE.2.1.TL10               | 67.46 ab        | 19.48       | 247.60 ab       | 25.94       |
| 17. | Streptomycine sulphate      | 60.66 b         | 7.44        | 190.40 abc      | -3.15       |
| 18. | Control                     | 56.46 bc        | 0.00        | 196.60 c        | 0.00        |

Means with the same letter are not significantly different by least significant difference at p < 0.05.

This study shown that endophyte strains inoculated to chili seedlings had greater value in all the growth parameter monitored (Table 2). It was determined that the bacterial endophyte as PGPR applications could be able to improve plant growth, seed germination rate, transplant emergence, response to stress condition, and protect from disease [30], such as Azospirillum, Pseudomonas and Azotobacter have significant impact on seed germination transplant growth [31, 32, 33]. The isolates of
bacterial endophyte improved seed germination and plant growth of oilseed rape and tomato significantly [34]. Our result showed, that bacterial endophyte treated chili seeds could increase the germination’s rate 19.00-42.85 % above the untreated seed (control). This result was higher than other research, such as: rhizobacterial strains enhanced seed germination of tomato up to 15 % over nontreated seed [35].

![Image](image_url)

Figure 3. Growth performance of bacterial endophyte introduced chilli (70 dat) (A). Control (B).

Bacterial endophyte introduced chili showed prolong the incubation period of wilt disease, reduce the disease incidence and severity (Table 3). This study demonstrates that 14 bacterial endophyte strains introduced chili could decrease of wilt disease incidence (0 %) in comparison to control plant (80 %) and without symptom until the end of the observations (70 DAT). This result confirms to our previous research on PGPR to control bacterial plant pathogens, such as 41 bacterial strains from healthy ginger rhizosphere from bacterial wilt disease endemic area could control the disease 100 % without symptom [28]. Yanti et al., (2017) reported that 13 rhizobacterial strains from rhizosphere of healthy chili could control bacterial wilt of chili 100 % and also without symptom [36]. The efficacy of those endophytic bacterial strains to control bacterial wilt disease and provided disease suppression equal or better compare with the other experiment, such as Wydra and Semrau (2005) reported comparable R. syzygii subsp. indonesiensis wilt disease reduction associated with biocontrol agents caused a significant reduction bacterial wilt disease on pepper and tomato compared to the control [37]. The protection afforded bacteria endophyte treated plants resulted no bacterial wilt symptom on chili. This suggested that bacterial endophyte treatment for some extent able to induced systemic resistance (ISR) of plant to overcome bacterial wilt infection on chili. Beneficial effects of bacterial endophyte as bioprotectants on plants have been reviewed. Bacteria endophyte may play many important beneficial roles in metabolism and physiology of the host plant [38], including to stimulate a latent disease defense mechanism as ISR, that confers an enhanced level of protection to a broad spectrum of pathogen [16].
Table 5. Effect of bacterial endophyte isolates on generative stage and yield of chili in pot experiment

| Nr  | Endophytic bacterial isolate | Generative stage | Yield |
|-----|-----------------------------|-------------------|-------|
|     |                             | Days after planting | Earlier (%) | g/plant | Ton/Hectare | Enhancement (%) |
| 1   | SLBE.1.1.AP1                | 57.40 ab           | -1.41        | 0.00* h  | 0.00        | -100.00        |
| 2   | SLBE.1.1.SN1                | 56.80 ab           | -0.35        | 55.44 e  | 1.85        | 27.19          |
| 3   | SLBE.2.1.BB2                | 48.00 a            | 15.19        | 61.53 d  | 2.05        | 41.16          |
| 4   | SLBE.3.1.AP3                | 52.20 ab           | 7.77         | 76.00 b  | 2.53        | 74.35          |
| 5   | SLBE.1.1.BB4                | 52.80 ab           | 6.71         | 68.30 c  | 2.28        | 56.69          |
| 6   | SLBE.2.3.BB4                | 59.00 ab           | -4.24        | 0.00* h  | 0.00        | -100.00        |
| 7   | SLBE.1.2.BB5                | 51.60 ab           | 8.83         | 32.26 g  | 1.08        | -25.99         |
| 8   | AGBE.1.1.TL5                | 55.40 ab           | 2.12         | 0.00* h  | 0.00        | -100.00        |
| 9   | AGBE.4.1.TL5                | 49.20 ab           | 13.07        | 74.80 b  | 2.49        | 71.60          |
| 10  | SLBE.2.2.AP6                | 60.00 b            | -6.01        | 53.26 e  | 1.78        | 22.18          |
| 11  | SLBE.3.3.AP6                | 57.40 ab           | -1.41        | 83.80 a  | 2.79        | 92.25          |
| 12  | SLBE.3.1.BB7                | 57.20 ab           | -1.06        | 64.89 cd | 2.16        | 48.86          |
| 13  | SLBE.4.2.BB8                | 49.20 ab           | 13.07        | 47.76 f  | 1.59        | 9.57           |
| 14  | AGBE.1.2.TL9                | 58.40 ab           | -3.18        | 35.59 g  | 1.19        | -18.35         |
| 15  | AGBE.3.1.TL9                | 59.60 ab           | -5.30        | 63.58 cd | 2.12        | 45.86          |
| 16  | AGBE.2.1.TL10               | 52.20 ab           | 7.77         | 44.83 f  | 1.49        | 2.84           |
| 17  | Streptomycin sulphate       | 55.40 ab           | 2.12         | 43.59 f  | 1.45        | 0.00           |
| 18  | Control                     | 56.60 ab           | 0            | 43.59 f  | 1.45        | 0.00           |

Means with the same letter are not significantly different by least significant difference at p < 0.05.

Most of endophytic bacterial strains could enhance growth and yield of chili (Table 4 and 5). The best endophytic bacterial strains to increase chili yield were SLBE3.1BB7 (83.80 g/plant and the enhancement 92.25 %), SLBE.3.1.AP3 (76 g/plant and the enhancement 74.35 %) and AGBE4.1.TL5 (74.80 g/plant and the enhancement 71.60 %). Different mechanisms are employed by endophytic bacteria to promote plant growth including both direct and indirect mechanisms. Direct mechanisms include facilitating nutrient uptake through nitrogen fixation, solubilization of phosphate, production of siderophores, production of phytohormones (such as auxins, cytokinins, and gibberellins), or production of the enzyme 1-aminoacyclopropane-1-carboxylate (ACC) deaminase [39]. In conclusion in the present study, three bacterial endophytic strains (SLBE.3.1.BB7, SLBE.3.1.AP3 and AGBE4.1TL) stands out as a possible candidate for use as biocontrol agent with plant growth promoting characteristics and these were isolated from healthy root of chili (indigenous). Hence it is proposed that potential strains observed in this study can be deployed as bioinoculants to increase the resistance of chili against R. syzygi subsp. indonesiensis and to promote growth and yield.

4. Conclusion

Three endophytic strains (SLBE.3.1.BB7, SLBE.3.1.AP3 and AGBE4.1TL) stands out as a possible candidate for use as biocontrol agent with plant growth promoting characteristics and these were isolated from healthy root of chili (indigenous). Hence it is proposed that potential strains observed in this study can be deployed as bioinoculants to increase the resistance of chili against R. syzygi subsp. indonesiensis and to promote growth and yield.
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