The ability of selected indigenous cyanobacteria isolates of West Sumatra to control *Fusarium oxysporum* f. sp. *capsici* on chili

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Abstract. The cyanobacteria provide a promising inoculant alternative for enhancement the growth, nitrogen fixation and yields. Our Previous study had screened the best cyanobacteria isolates, which has potential as a plant growth promoter. However, the ability of the cyanobacteria strain to control Fusarium wilt disease is not yet identified. This research aimed to screen the best cyanobacteria strains isolated from chili rhizosphere as growth promoter and biocontrol agents for fusarium wilt on chili. Cyanobacteria multiplied with common methods using BG-11 medium (room temperature, 12/12 h L/D cycle). All strains showed ability as growth promoter and increase yields. All isolates also showed suppression of disease development caused by *Fusarium oxysporum* f.sp. *capsici* (Foc). All the five strains that are known for their ability to control *R. syzygii* subsp. *indonesiensis* were also had the best ability to promote growth and increase resistance to Foc.

1. Introduction

*Capsicum annuum* L. is an economically important crop consumed as spicy substitution or ingredient in the world [1]. Due to its high value and consumption rate, the annual trade of chilies reaches approximately 17% of total spicy ingredient trades in the world [2]. However, chili productions can be decreased due to pathogen attack such and *Fusarium oxysporum* f. sp. *capsici*, the main causal agents of fusarium wilt disease. The disease has consistently caused an annual yield loss of about 10-80% [3].

Biofertilizers and antagonist activity by microbial usage had provides a promising alternative to chemical fertilizers and pesticides. Interactions of plants and microbes can influence plant growth promotion activity, regulate nutrient absorption, and alter plants' abiotic/biotic stress response [4]. Plant growth-promoting rhizobacteria (PGPR) that colonize plant roots and enhance plant growth by a wide variety of mechanisms [5]. PGPR usage in agriculture was steadily increasing due to its attractive way of reducing agrochemical usages such as pesticides and fertilizers.

Inoculation of cyanobacterial (blue-green algae) has been known for promoting the growth, nitrogen fixation, and increase the yields in the rice-wheat cropping sequence [6]. Cyanobacteria are prominent inhabitants of many agricultural soils, such as rice roots [7-8]. The cyanobacteria
potentially contribute in fixating nitrogen [9], solubilizing phosphate [10] and other mineral release that increase the soil fertility and crop productivity [11].

Inoculation of cyanobacterial is known to enhance the growth, nitrogen fixation and yields of crops [6, 12-13]; however, very few reports are available on their role in a disease reduction and protection against fungal diseases [14-15]. The reports had shown the ability of cyanobacteria to promote growth and yields. Although, their role in plant growth promotion and soil microbial activity is still not well-known. Our Previous study had screened five best cyanobacteria isolates, which has the potential to plant growth promotion [16]. However, the ability of these strain to control Fusarium wilt disease is not yet identified. This research purposed to screen best cyanobacteria from the chili rhizosphere to promote growth rate and control fusarium wilt of chili.

2. Materials and methods
2.1. Assay of indigenous cyanobacteria to control Fusarium wilt
All five isolates to assay the strains' ability to control Fusarium oxysporum f.sp. capsici. Research conducted in two separates experiments both for each pathogen. Research done in completely randomized designs with five replications with six treatments (5 strains of indigenous cyanobacteria and control).

2.2. Multiplication of cyanobacterial isolates
All isolates used for this study had been collected in microtube 1.5 mL. All isolates were recultured in BG-11 medium agar in a petri dish with striking the collection to the growth of the plate in 2 days in an incubator (L/D cycles 16:8 h, 50–55 mmol photons m, 27°C) [17]. The growth culture then regrowth on the same methods and incubated for 48 hours. The pure colony growth in the plates was used for further study. The selected isolates multiplied with modified methods of Yanti et al. [16] using BG-11 and incubated according to the same parameters with the streaked method.

2.3. Inoculation of indigenous cyanobacteria on chili
Chili seeds (Var. Laris) surface sterilized before used with common methods using aqua dest and NaOCl 1%. Sterilized chili seeds dipped to indigenous cyanobacteria (25 replications) suspensions for 10 minutes, wind-dried and planted to portray contain sterilized soil mixture (soil and organic manure 2:1 v/v ) (control dipped in sterilized water) and growth for 3 weeks. The germination rate, seedlings' height, and the number of leaves on this stage were also observed. All chili seedlings' introduced with indigenous cyanobacteria growth in seeding then planted to polybag contain soil and organic manure (2:1 v/v) [18]. Seedlings planted in triplications and introduced with the same indigenous cyanobacteria isolates with dipping methods.

2.4. Inoculation of Fusarium oxysporum f. sp. capsici (Foc)
Foc was acquired from diseased plants, isolated and cultured on Potato Dextrose agar. Foc inoculated one week before planting with 10 gr of Foc cultured in rice (1 week old). Parameter observed in this research is incubation time, incidence, severity, Plant height, number of leaves, and yields. All parameters were subjected to ANOVA in accordance with completely randomized experimental design and furtherly assayed using LSD test at P<0.05.

3. Results and discussions
The introduction of cyanobacteria isolates to chili seeds can increase field germinability compare to control. All cyanobacteria isolates could increase chili seedlings' height and germination (compared with control). The total of leaves also showed varied but also shown higher than control (Table 1).
Cyanobacteria isolates CBY 3.1.3 were shown the highest parameter among all indigenous endophyte isolates assayed with seedlings' height 9.633 cm and germination rate 100%.

Table 1. Chili seedlings growth introduced with cyanobacteria isolates (21 DAI)

| Isolates  | Germination rate (%) | Seedling's height (cm) | Total of Leaves |
|-----------|----------------------|------------------------|-----------------|
| CBY 3.1.3 | 100.00 a             | 9.633 a                | 8.00 a          |
| CBY 5.1   | 99.333 ab            | 9.567 b                | 7.67 b          |
| CBY 44    | 99.333 ab            | 9.467 c                | 7.67 b          |
| CBY 9.1.3 | 96.000 b             | 9.367 cd               | 7.00 c          |
| CBY 2.3.1 | 96.000 b             | 9.367 cd               | 6.33 b          |
| Control   | 95.333 bc            | 9.300 d                | 5.33 e          |

Cyanobacteria isolate introduction to the chili seedlings also shown an increased growth rate compared to untreated seedlings. The introduction of cyanobacteria isolates also showed a higher promotion rate of generative stage on chili as shown in Table 2. All isolates showed increased growth in plant height, the number of leaves, and also generative parameters such as promoting flowering time and increase yields of chili plants. Isolates CBY 3.1.3, which were the best strains to promote growth rate of chili on the seedling phase also shown best ability to promote plant height (107.00 cm), number of leaves (43.00), the first day of flowering (26.0 days after planting) and yields (1,102.4 g).

Table 2. Plant growth promotion activity on generative phase of chili by Cyanobacteria introduction.

| , Isolates  | Plant height (cm) | Number of leaves | First flowering day | Yields (g) |
|-------------|-------------------|------------------|---------------------|------------|
| CBY 3.1.3   | 107.00 a          | 43.00 a          | 26.00 a             | 1,102.4 a  |
| CBY 5.1     | 97.00 ab          | 36.00 b          | 28.67 ab            | 1024.7 b   |
| CBY 44      | 94.33 b           | 35.33 b          | 30.67 b             | 989.33 bc  |
| CBY 9.1.3   | 88.00 c           | 35.33 b          | 33.33 c             | 812.67 c   |
| CBY 2.3.1   | 77.00 d           | 22.67 c          | 42.00 d             | 671.33 d   |
| Control     | 60.67 c           | 18.67 d          | 43.33 d             | 316.23 e   |

Cyanobacteria isolates introduction had shown decrease disease activity (Table 3). All isolates shown the ability to decrease disease incidence up to 0% and shown no symptoms of Fusarium wilt, furthermore increase growth on the generative phase (after pathogen inoculation). Isolates CBY 3.1.3 have both the highest ability to promote the growth rate of chili and control Fusarium wilt diseases.

Table 3. Disease development of Fusarium wilt of chili pepper introduced with indigenous endophyte bacteria

| Isolates  | Disease development time | Effectivity | Disease Incidence (%) | Effectivity | Severity | Effectivity |
|-----------|--------------------------|-------------|-----------------------|-------------|----------|-------------|
| CBY 3.1.3 | 42.00                     | 106.59      | 0.00                  | 100.00      | 0.00     | 100.00      |
| CBY 5.1   | 39.33 ab                 | 93.46       | 66.67                 | 33.33       | 0.67 ab  | 83.25       |
| CBY 44    | 31.00 f                   | 52.48       | 66.67                 | 33.33       | 2.67 ab  | 33.25       |
| CBY 9.1.3 | 32.67 bc                  | 60.70       | 100.00                | 0.00        | 2.00 bc  | 50.00       |
| CBY 2.3.1 | 30.67 c                   | 50.86       | 66.67                 | 33.33       | 2.67 ab  | 33.25       |
| Control   | 20.33 d                   | 100         | 4.00                  |             |          |             |
Plant rhizosphere is a rich habitat for soil microorganisms complex [19] like PGPR, which stimulates plant growth by colonizing root, synthesizing hormones such as IAA, and mobilizing nutrients [20]. PGPR are known to increase the availability of nutrients in the rhizosphere, which may be through solubilisation of unavailable forms of nutrients and/or siderophore production and facilitating transport of nutrients [21]. Our study found that most of the selected cyanobacteria screened from the first stage were able to promote growth and increase yields of chili.

We conclude that the cyanobacteria screened from this current study have the ability to promote growth rate and yields of chili mechanisms by enhancing nutrients uptake for the plants. Researchers reported that plant growth-promoting rhizobacteria, including cyanobacteria can enhance plant height and productivity by synthesizing phytohormones, thereby increasing the local availability of nutrients or facilitating the uptake of nutrients [22-24]. There is conclusive evidence that nitrogen fixed by cyanobacteria is made available to the plants or other micro/macro-organisms in the surrounding environment [25].

Interactions of plant and bacteria, including cyanobacteria, had been reported [26-28]. Efficient colonization of microbes known as related to increased nutrients available on the root surface or to the root exudation[29]. The interaction of cyanobacteria was also reported on peas[30]. This is supported by earlier studies using cyanobacteria which revealed their significant role in nutrient cycling and biofortification of wheat crop [5, 13, 31].

4. Conclusion
All cyanobacteria isolates had the ability to promote the growth rate and increase yields. CBY.3.13 cyanobacteria isolates were the best isolates to promote growth rate and increase yields of chili. Thus the isolates were best candidates for further development of novel growth promotor. We need further study to determine their ability as biocontrol agents for other pathogens.

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