

Screening of Indigenous Rhizospheric Cyanobacteria as Potential Growth Promotor and Biocontrol of *Ralstonia syzygii* subsp. *indonesiensis* on Chili

Yulmira Yanti¹, Hasmiandy Hamid¹, Zulfadly Syarif²

¹Department of Plant Protection, Faculty of Agriculture, Andalas University, Padang, West Sumatra, Indonesia

²Department of Agrotechnology, Faculty of Agriculture, Andalas University, Padang, West Sumatra, Indonesia

Abstract— The use of microbial inoculants as biofertilizers and/or antagonists of phytopathogens provides a promising alternative to chemical fertilizers and pesticides. Cyanobacteria are a remarkable group of prokaryotes, which are known to exist independently and in symbiotic/facultative associations with a diverse range of members of the plant. Cyanobacteria inoculation had been reported to enhance the growth, nitrogen fixation and yields. Although, their establishment and role in plant growth promotion and biocontrol activity is poorly known. This research purposed to isolate and screen the best indigenous cyanobacteria from chili rhizosphere to promote growth rate and control *R. syzygii* subsp. *indonesiensis* on chili. The study consisted of three parts: (i) isolation and multiplication of the cyanobacteria from soil rhizosphere, and screening through Hypersensitive response (HR) on *Mirabilis jalapa* for pathogenicity test, (ii) in planta screening of selected cyanobacteria isolates (from second's step) to increase growth of chili seedlings, and (iv) in planta evaluation of selected cyanobacteria isolates for the control of bacterial wilt disease and promote growth of chili. Cyanobacteria isolated with BG-11 medium and incubated in room temperature with 12/12 h light/dark cycle. 49 cyanobacteria had isolated from chili rhizosphere. All isolates also showed suppression of disease development caused by *R. syzygii* subsp. *indonesiensis*. BCBY 3.1.3, and CBY 5.1 showed suppression of symptom appear (60.00 day post inoculation (dpi) compared to control (.38.667 dpi) and also suppressed disease severity (1.67) compared to control (3.00). The two strains which have best ability to increased growth rate also have best ability to fully suppressed disease development with no symptom appear until last day of observation.

Keywords— Cyanobacteria; PGPR; screening; chili;

I. INTRODUCTION

Ralstonia syzygii subsp. *indonesiensis* [1], previously named *Ralstonia solanacearum* phylotype IV, is a soil-borne gram-negative bacterium that causes bacterial wilt disease in over 200 families of plants, including chili [2,3]. This pathogen causes wilt by infecting plants through roots and colonizing stem vascular tissue and the vascular tissues in the lower stem of the wilted plants usually show a brown discoloration [4]. Attention has been paid to minimize the disease infestation through cultural practices, development of resistant varieties and use of chemicals, but most of them have a limited success [5].

Current trends in agriculture are focused on the reduction of the use of pesticides and inorganic fertilizers, forcing the search for alternative ways to improve crop yield in sustainable agriculture [6]. Biological systems are

therefore preferred over chemical fertilizers, as they are not only ecofriendly and economical in approach, but are also involved in improving the soil quality and maintenance of natural soil flora.

The use of microbial inoculants as biofertilizers and/or antagonists of phytopathogens provides a promising alternative to chemical fertilizers and pesticides. Plant microbe interactions involve beneficial and detrimental relationships and the type of microorganisms involved determines the final outcome of the relationship, ranging from pathogenesis to symbiosis. Such interactions can influence plant growth and development, modulate nutrient dynamics, and alter a plant's susceptibility to abiotic /biotic stress [7]. Plant growth promoting rhizobacteria (PGPR) include beneficial bacteria that colonize plant roots and enhance plant growth by a wide

variety of mechanisms [8]. The use of PGPR is steadily increasing in agriculture, as it offers an attractive way to reduce the use of chemical fertilizers, pesticides and related agrochemicals.

Cyanobacteria are cosmopolitan microorganisms, which play significant roles in diverse ecosystems. Cyanobacterial inoculation is known to enhance the growth, nitrogen fixation and yields in the rice-wheat cropping sequence [9]. The favorable conditions provided by the rice fields for nitrogen fixation by these organisms leads to enhanced plant-available N in soil and yield improvement of rice [10, 11]. Cyanobacteria liberate extracellular substances and modulate pH, temperature and redox activity, besides playing a role in the volatilization of ammonia and methane generation; therefore, are directly or indirectly implicated in the management and productivity of rice ecosystem [12]. Cyanobacterial inoculation is also known to improve the stability of soil due to excretion of polysaccharides, lipids which aid in enhancing aggregation [13]. Their influence on other crops besides rice, e.g. wheat, tomato, chili and pulse and vegetable crops were also documented [14-16].

Cyanobacteria are potentially contributed towards biological nitrogen fixation [17], phosphate solubilization [18] and mineral release to improve soil fertility and crop productivity [19]. Cyanobacteria were also known to add organic matter, synthesize and liberate amino acids, vitamins and auxins, reduce oxidizable matter content of the soil, provide oxygen to the submerged rhizosphere, ameliorate salinity, buffer the pH, solubilize phosphates and increase the efficiency of fertilizer use in crop plants [20-21].

Cyanobacterial inoculation is known to enhance the growth, nitrogen fixation and yields in the rice-wheat cropping sequence [22, 23]; however, very few reports are available on their role in disease reduction and protection against fungal diseases [24,25]. Cyanobacteria inoculation had been reported to enhance the growth, nitrogen fixation and yields. Although, their establishment and role in plant growth promotion and biocontrol activity is poorly known. This research purposed to isolate and screen the best indigenous cyanobacteria from chili rhizosphere to promote growth rate and control *R. solygyii* subsp. *indonesiensis* on chili.

II. METHODS

2.1. Study Area

This research has been done in Laboratory of Microbiology, Department of Plant Protection, and greenhouse, Faculty of Agriculture, Andalas University, Padang, Indonesia during January to July 2019.

2.2. Procedures

The study consisted of three parts: (i) isolation and multiplication of the cyanobacteria from soil rhizosphere, and screening through Hypersensitive response (HR) on *Mirabilis jalapa* for pathogenicity test, (ii) in planta screening of selected cyanobacteria isolates (from second's step) to increase growth of chili seedlings, and (iv) in planta evaluation of selected cyanobacteria isolates for the control of bacterial wilt disease and promote growth of chili.

2.3. Isolation of potential cyanobacteria isolates

Rhizosphere samples were collected from healthy chili's rhizosphere in endemic area of bacterial wilt in Tanah Datar, Solok and Agam District, West Sumatra Province, Indonesia. Soil and plant samples were given identification tags indicating the location, date of collection and type of crop, and were brought to the Microbiological Laboratory at Faculty of Agriculture, Universitas Andalas, Padang, West Sumatra, Indonesia. Samples were isolated one day after transport to the laboratory.

1 g of rhizospheric soil sample of chili were homogenized with 10 mL of sterilized tap water and serially diluted up to 10^{-5} . From this suspension, 1 mL of the suspension then suspended separately to 10 mL of BG-11 media, Yeast extract mannitol agar (YEMA) and Jensen's Medium agar and then separately placed in a Petri dish and growth in 2 days at incubated at 27 °C in an incubator with light/dark cycles (16:8 h) with white light (50–55 mmol photons m⁻² s⁻¹) [26]. Thereafter, dominant bacterial colonies were purified on the previous growth medium. A single colony of bacteria then transferred aseptically to microtube that contain 1 mL of sterilized aquadest as stock and stored in refrigerator. The isolates of cyanobacteria morphological character were noted based on morphological character of colonies, gram test and hypersensitive reactions (HR) assayed all according to methods of Klement et al., [27]. The positive results of HR indicated that the assayed isolates were pathogen to plants and not used for further studies.

2.4. In planta screening of selected cyanobacteria isolates to increase growth of chili seedlings

The selected isolates multiplied with preculture and main culture in BG-11 medium according to modified methods of Yanti et al., [28], and its density adjusted to 10^8 CFU/mL with McFarland solutions scale 8.

Chili seeds used are varieties of Laris. Seeds sterilized before used with consecutively sterilized aquadest, NaOCl 1%, three times rinsed with sterilized water each for 2 minutes and then wind dried.

Sterilized chili seeds dipped to cyanobacteria suspensions and control dipped to sterilized aquadest for 10 minutes, wind dried and planted to pot-tray contained sterilized soil and cow dung manure mixture (2:1 v/v). Nurseries done in 3 weeks with parameter observed were germination rate, seedlings' height and number of leaves. All treatments used 25 seeds.

2.5. In planta evaluation of selected cyanobacteria isolates to promote growth and yield of chili

All chili seedlings from previous stage were planted to polybag contain same soil mixtures and reintroduced with cyanobacteria isolates with dipping methods for 15 minutes before. Parameter observed height, number of leaves, first flowering and yields.

III. RESULTS AND DISCUSSIONS

The cyanobacterial isolates were collected from chili field area located in Solok, Tanah Datar and Agam Regency, West Sumatra Province, Indonesia. The strains were purified and characterized morphologically and physiologically as given in Table 1. For morphological characterization of the plant growth promoting cyanobacterial were recorded. From 49 cyanobacteria isolates, 10 isolats were found to had positive results to Hypersensitive (HR) test. The HR results indicate the possibilities of the isolates as plant pathogens and were not used further.

Table 1. Indigenous Cyanobacteria isolated from Rhizosphere of Chili

No	Isolates	Gram	HR	Colony Shape				
				Colour	Shape	Elevation	Margin	Size (cm)
1	CBY 1.2.1	+	+	Yellowish cream	Circular	Convex	Entire	0.1
2	CBY 1.2.2	+	-	white	Circular	Convex	Entire	0.2
3	CBY 1.3	+	+	white	Irregular	Umbonate	Undulate	0.2
4	CBY 2.2.1	+	-	Yellowish cream	Circular	Convex	Entire	0.2
5	CBY 2.2	+	-	Yellowish cream	Circular	Convex	Entire	0.2
6	CBY 2.3.1	+	-	white	Irregular	Umbonate	Undulate	0.2
7	CBY 2.3.2	+	-	Yellowish cream	Circular	Convex	Entire	0.2
8	CBY 2.2.2	+	-	Yellowish cream	Circular	Convex	Entire	0.2
9	CBY 3.2	+	-	Yellowish cream	Circular	Convex	Entire	0.2
10	CBY 3.1.3	+	-	Yellowish cream	Circular	Convex	Entire	0.3
11	CBY 3.3	-	-	brown	Irregular	Umbonate	Undulate	0.4
12	CBY 3.1.3	+	-	Yellowish cream	Circular	Convex	Entire	0.1
13	CBY 3.2	+	-	brown	Irregular	Umbonate	Undulate	0.4
14	CBY 3.3.3	-	+	Yellowish cream	Circular	Convex	Entire	0.4
15	CBY 4	+	-	greenish	Circular	Convex	Entire	0.3
16	CBY 4.2	+	-	white	Irregular	Umbonate	Undulate	0.2
17	CBY 44	+	-	Yellowish cream	Circular	Convex	Entire	0.2
18	CBY 5.2	+	+	white	Irregular	Umbonate	Undulate	0.6
19	CBY 5.1	+	-	greenish	Circular	Convex	Entire	0.3
20	CBY 6.1.3	+	+	white	Irregular	Umbonate	Undulate	0.3
21	CBY 6.1.1	+	-	Yellow	Circular	Convex	Entire	0.3
22	CBY 6.1.2	+	-	Yellowish cream	Circular	Convex	Entire	0.1
23	CBY 7.1	+	-	Yellowish cream	Circular	Convex	Entire	0.1
24	CBY 7.2	-	-	white	Circular	Convex	Entire	0.2
25	CYB 2.5.2	-	-	Yellowish cream	Circular	Convex	Entire	0.3
26	CYB 4.3	-	-	grey	Circular	Convex	Entire	0.1
27	CYB 5.3	+	-	grey	Circular	Convex	Entire	0.1
28	CYB6.4	+	-	Yellowish cream	Circular	Convex	Entire	0.2
29	CYB 7.3	+	+	white	Circular	Convex	Entire	0.5
30	CYB 7.4	+	-	white	Circular	Convex	Entire	0.5
31	CBY 8.2.2	+	-	greenish	Circular	Convex	Entire	0.4
32	CBY 8.2.1	+	-	greenish	Circular	Convex	Entire	0.3
33	CBY 8.2.3	+	-	greenish	Circular	Convex	Entire	0.2

34	CBY 9.1.3	+	-	Grey	Irregular	Raised	Undulate	0.1
35	CBY 10.1.1	+	-	Yellowish cream	Circular	Convex	Entire	0.2
36	CBY 10.2.1	+	+	Yellowish cream	Circular	Convex	Entire	0.1
37	CBY 10.1.2	+	-	Yellowish cream	Circular	Convex	Entire	0.3
38	CBY 10.2.2	+	-	Yellowish cream	Circular	Convex	Entire	0.3
39	CYB 8.3	+	-	grey	Circular	Convex	Entire	0.2
40	CYB 8.4	+	-	yellow	Circular	Convex	Entire	0.2
41	CYB 9.3.1	+	-	white	Circular	Convex	Entire	0.2
42	CYB 9.4	+	-	Yellowish cream	Circular	Convex	Entire	0.3
43	CYB 9.3	+	-	white	Circular	Convex	Entire	0.2
44	CYB 9.3.2	+	+	white	Circular	Convex	Entire	0.2
45	CBY 11.2.2	+	-	Yellowish cream	Irregular	Umbonate	Undulate	0.4
46	CBY 11.1.1	+	-	Yellowish cream	Circular	Convex	Entire	0.1
47	CBY 11.2.1	+	-	Yellowish cream	Circular	Convex	Entire	0.1
48	CBY 11.1.2	-	+	Yellowish cream	Circular	Convex	Entire	0.1
49	CBY 12.2	-	+	Yellowish cream	Circular	Convex	Entire	0.1

Ten of 39 cyanobacteria isolated from chili rhizosphere could increase height and germination rate of chili seedlings compared to control. Total of leaves also showed varied (Table 1). CBY 3.1.3 were the best isolates among all the cyanobacteria assayed with seedlings' height 11.06 cm and total leaves 5.33 with effectivity 105%. The 10

cyanobacteria strains (CBY 3.1.3, CBY 5.1, CBY 9.1.3, CBY 3.2, CBY 44, CBY 4.2, CYB 9.4, CBY 2.3.1, CBY 10.2.2 and CBY 3.3) were the best isolates to promote growth rate (seedlings' height and total of leaves) of chili and used for further studies.

Table 2. Height and total leaves of seedlings introduced with cyanobacteria isolates (21 days after introduction (DAI))

Isolates	Seedlings' height (cm)	Total of leaves
CBY 3.1.3	11.10 a	5.33 a
CBY 5.1	10.30 ab	5.00 ab
CBY 9.1.3	10.23 ab	5.00 ab
CBY 3.2	9.50 bc	5.33 a
CBY 44	9.50 bc	5.33 a
CBY 4.2	9.27 bcd	5.33 a
CYB 9.4	8.67 cde	5.00 ab
CBY 2.3.1	8.63 cde	4.33 bc
CBY 10.2.2	8.50 cdef	5.00 ab
CBY 3.3	8.40 cdef	4.33 bc
Control	8.20 cdefg	4.66 abc
CBY 2.2.2	8.20 cdefg	5.00 ab
CYB 4.3	8.16 cdefgh	4.66 abc
CYB 9.3.1	8.13 cdefgh	4.66 abc
CBY 4	8.00 cdefghi	4.66 abc
CYB 8.4	8.00 cdefghi	5.00 ab
CYB 5.3	7.93 defghi	4.33 bc
CBY 2.3.2	7.90 defghi	4.33 bc
CYB 8.3	7.90 defghi	4.66 abc
CBY 6.1.1	7.83 defghi	4.33 bc
CBY 8.2.3	7.80 defghi	4.33 bc

CBY 10.1.2	7.66 efghi	5.00 ab
CBY 2.2	7.63 efghij	4.00 c
CBY 2.2.1	7.53 efghijk	4.66 abc
CBY 3.1.3	7.50 efghijk	4.33 bc
CBY 3.2	7.36 efghijkl	4.33 bc
CBY 11.2.1	7.33 efghijklm	4.66 abc
CBY 10.1.1	7.20 efghijklmn	4.33 bc
CBY 8.2.1	7.16 efghijklmn	4.33 bc
CYB 2.5.2	7.06 fghijklmn	4.33 bc
CBY 1.2.2	7.03 fghijklmn	4.33 bc
CBY 7.2	7.03 fghijklmn	4.00 c
CYB 9.3	6.73 ghijklmn	4.00 c
CBY 6.1.2	6.66 hijklmn	4.00 c
CBY 11.1.1	6.56 ijklmn	4.33 bc
CYB6.4	6.13 jklmn	4.00 c
CBY 8.2.2	6.10 klmn	4.66 abc
CYB 7.4	5.93 lmn	4.33 bc
CBY 11.2.2	5.83 mn	4.00 c
CBY 7.1	5.73 n	4.00 c

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

Phosphorus and nitrogen are very essential nutrients for plant growth and inoculation with phosphate solubilizing and nitrogen fixing cyanobacteria has been shown to improve plant growth by increasing the availability of phosphate and nitrogen content [29-31]. Cyanobacterial strains may protect plants from phytopathogens due to hydrogen cyanide production [32]. Phytohormone producing cyanobacteria are also involved in the promotion of plant growth. Need further checked for to determined cyanobacteria with ability to promote growth.

Beneficial effect of cyanobacteria introduction in chili plants were also noticed in generative stage of chili growth. The result proved that 7 of 10 isolates was able to increase growth of chili plants compared to control (Table 3). The strains also promote flowering time and increase yields of chili. The 10 best cyanobacteria strains were CBY 3.1.3, CBY 5.1, CBY 9.1.3, CBY 3.2, CBY 44, CBY 4.2 and CYB 9.4.

Table 3. Plant growth promotion activity of cyanobacteria isolates on generative phase of chili.

Isolates	Plant height (cm)	Total of leaves	First flowering time	Yields (g)
CBY 3.1.3	76.667 a	20.000	44.500 a	168.32 a
CBY 5.1	75.667 a	19.000	43.500 a	145.12 b
CBY 9.1.3	73.333 a	19.000	42.600 ab	141.60 b
CBY 3.2	70.000 a	19.000	41.200 ab	135.29 bc
CBY 44	66.000 ab	18.667	41.200 ab	129.78 c
CBY 4.2	62.000 ab	17.333	40.000 b	124.63 c
CYB 9.4	60.667 ab	16.667	38.600 c	115.26 d
control	59.000 ab	16.667	36.400 cd	113.44 d
CBY 2.3.1	58.000 ab	15.667	34.600 d	108.46 de
CBY 10.2.2	58.000 ab	15.000	34.400 d	93.988 e
CBY 3.3	50.333 b	14.000	33.000 de	86.452 f

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

The increase in shoot lengths and number of leaves on chili plants, and furthermore the yields could be due to the action of one or more of the growth promoting substances especially seeds with cyanobacterial cultures. The results of inoculated and non-inoculated plants were recorded in order to make comparison. Many researchers have reported that the co-cultivation of crops with cyanobacteria caused a considerable increment in growth and biochemical parameters, both in control and field conditions [33-35].

Another study also found that the cyanobacterial inoculations could also promote growth rate of plants such as wheat [16, 38], *Lupinus termis* [37], Pea [34] and rice [38,39]. The phyto-stimulatory potential of cyanobacteria was also attributed to the atmospheric nitrogen fixation, making it available to the associated plants [16]. Increase

in growth affects the overall development and growth of the plants by stimulating the water and nutrient uptake from soil. From the present study, we concluded that both cyanobacterial strains stimulated the growth of plant and they can be effectively used for biofertilization and plant growth improvement of different crops.

All isolates also showed suppression of disease development caused by *R. syzygii* subsp. *indonesiensis*. BCY 3.1.3, and CBY 5.1 showed suppression of symptom appear (60.00 day post inoculation (dpi) compared to control (.38.667 dpi) and also suppressed disease severity (1.67) compared to control (3.00). the two strains which have best ability to increased growth rate also have best ability to fully suppressed disease development with no symptom appear until last day of observation.

Table 4. *R. syzygii* subsp. *indonesiensis* disease development on chili plant inoculated with Cyanobacteria Indigenos

Isolates	First Symptom Developed	Severity
CBY 3.1.3	60.000 a	1.67 a
CBY 5.1	60.000 a	1.67 a
CBY 44	50.667 ab	1.67 a
CBY 9.1.3	49.000 abc	1.33 ab
CBY 2.3.1	43.000 abc	1.00 abc
control	38.667 abc	0.67 abc
CYB 9.4	37.333 bc	0.67 abc
CBY 3.2	36.333 bc	0.33 bc
CBY 4.2	32.000 bc	0.00 c
CBY 10.2.2	29.667 bc	0.00 c
CBY 3.3	27.667 c	0.00 c

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

Cyanobacterial strains showed biocontrol activity against *R. syzygii* subsp. *indonesiensis*. However further studies about their mechanisms in in vitro conditions were necessary to know the isolates abilities. The potential activity of cyanobacteria to inhibit certain soilborne diseases such as *R. syzygii* subsp. *indonesiensis* needs further investigation before they can be accepted as biocontrol agents for agriculture. Although the reports of cyanobacteria ability as biocontrol were not much, cyanobacterial strains had been reported to produce a wide range of plant growth regulators such as abscisic acid, ethylene, jasmonic acid, auxin, and cytokinin-like substances as well as the cytokinin isopentenyl adenine [39,40]. The cyanobacteria abilities as biocontrol agents of plant pathogens were still not well studied. Other have been evaluated the antifungal activity of terrestrial cyanobacterium *Nostoc commune* against the *Candida albicans* [41,42]. These antifungal activities are very interesting in the perspective of cyanobacterial research

and possibly are important in commercial. Nevertheless, the antifungal activities of cyanobacterial metabolites were rarely studied.

IV. CONCLUSION

From the present study, we concluded that the cyanobacterial isolated from Chili Rhizosphere in West Sumatera could stimulated the growth of chili plant, Increase yields and control *R. syzygii* subsp. *indonesiensis*. Out of all the strains assayed in this study, isolates CBY 3.1.3 and CBY 5.1. were the best isolates to both promote growth, increase yields and control *R. syzygii* subsp. *indonesiensis*. Further studies are need to determine the strains ability.

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