Effectivity of *Bacillus cereus* to control *Ralstonia syzygii* subsp. *indonesiensis* and growth promoting of chili pepper

Yulmira Yanti*, Warnita, Reflin, and Chainur Rahman Nasution

ABSTRACT

Four *Bacillus cereus* isolates acquired from previous studies have good biocontrol and plant growth-promoting activity in plants. This study purposes to acquire best *Bacillus cereus* which has the ability to control *Ralstonia syzygii* subsp. *indonesiensis* and plant growth promoting activity on fields. Research was conducted in Alahan Panjang, Solok District, West Sumatera, Indonesia. It used Complete Randomized Design with three replications. Parameters observed are disease development (Symptom, severity), growth development (germination rate, plant height, number of leaves, root length) and generative phase (Time of flowering and yields). Result showed that indigenous rhizobacterial isolates have varied ability to control pathogen and promote growth. All *B. cereus* persist in moderate density after 30 week introduction and have the ability to control disease development and reduce the symptom appearance of *Bacillus cereus* Strain JN233 have the ability to control *R. syzygii* subsp. *indonesiensis* and have best ability to promote growth rate of pepper.

Keywords : *Bacillus cereus*, *Ralstonia R. syzygii* subsp. indonesiensis, Plant Growth Promoting Rhizobacteria.

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INTRODUCTION

Ralstonia syzygii subsp. *indonesiensis* (Safni *et al*., 2014), formerly named *R. solanacearum* (Yabuuchi *et al.*, 1995) (RS) is a gram-negative, soil-borne pathogen that causes bacterial wilt (BW), a disease affecting more than 50 plant families (Hayward, 1994). This disease is economically important for several vegetable crops including members of the Solanaceae family such as pepper (*Capsicum annuum* L.) (Hartman and Elphinstone, 1994). Bacterial Wilt caused by *R. syzygii* subsp. *indonesiensis* is one of the important serious vascular diseases of chilli crop causing maximum crop losses (Basu, 2014). Bacterial wilt causes 15% to 55% crop losses around the world (El-Argawy and Adss, 2016).

The use of beneficial microorganisms could be an environmentally sound option to increase crop yields and reduce disease incidence (Calvo *et al.,* 2010). Plant growth-promoting rhizobacteria (PGPR) colonize plant roots and induce an increase in plant growth (Vessey, 2003). Among the mechanisms by which PGPR exert beneficial effects on plants are facilitating the uptake of nutrients such as phosphorus via phosphate solubilization, synthesizing stimulatory phytohormones like indole-3-acetic acid (IAA) (Vessey, 2003), or aiding in the control of the deleterious effects of pathogens by producing inhibitory substances, excluding them from the roots by competition or by inducing systemic resistance (Compant *et al.,* 2005). The most common PGPR reported are strains of the genus Bacillus (Compant *et al.,* 2005; Vessey, 2003). *Bacillus* spp. are considered to be safe microorganisms that hold remarkable abilities for synthesizing a vast array of beneficial substances (Stein, 2005). *Bacillus* strains have the advantage of being able to form endospores which confers them high stability as biofungicides or biofertilizers (Schisler *et al.,* 2004). *Bacillus* spp. having potent growth promoting traits such as IAA production, phosphate solubilization, nitrogen fixation,

and biocontrol attributes like production of HCN, siderophore, hydrolytic enzymes and antibiotics have been isolated (Senthilkumar *et al.*, 2009). Members of the Bacillus genus are often considered microbial factories for the production of a vast array of biologically active molecules potentially inhibitory for phytopathogens growth (Ongena and Jacques, 2008).

MATERIAL AND METHODS

This Research was done from March to September 2017 in the Microbiology Laboratory, Department of Plant Protection, Faculty of Agriculture, Andalas University, Padang, Indonesia.

Multiplication of Isolates

Isolates collection from microtubes are grown on Nutrient agar and incubated for 3 days. One pure colony of isolates is added to 25 mL of NB in culture bottle (50mL) and incubated in rotary shaker for 24 hrs. 1 mL preculture transferred to 150 mL of sterile coconut water in Erlenmeyer flask for mainculture and incubated for 2 x 24 hrs (Yanti and Resti, 2010). Suspension of rhizobacteria from mainculture is diluted with comparison to McFarland scale 8 (Density estimated 108 CFU/mL) (Yanti *et al.*, 2013).

Seeding and planting

Seeding of chili seed is done in seedbed. Seed is introduced with *Bacillus cereus* strains with dipping method in rhizobacteria suspension with density 108 CFU/mL. Seeding is done in 21 days. Chili seedlings are then planted in sterilized soil (2:1 v/v soil: cow dung manure (Yanti *et al*., 2013) with reintroduction with *B. cereus* strains isolates following the same method. *R. syzygii* subsp. *indonesiensis* inoculated 2 weeks after planting. Parameter Observed are Growth rate (Germination Rate, Plant Height, Total Leaves, Root Length, Flowering Time and Yields on Chili Plants) and disease development (symptoms appearance and severity). Plant Growth Promoting Rhizobacteria Attributes of *Bacillus cereus* strains

Indole Acetic Acid (IAA) Production

IAA from *B. cereus* strains are remained with Calorimeter method by Bric *et al.* (1991). Bacteria is Cultured in King's B broth medium and incubated on shaker (200 rpm 48 hrs). Bacterial culture is then centrifuged in 7000g for 15 minutes. Supernatant is separated from pellets, 2 mL of supernanant added to 4 mL salkowsky reagent (1mL FeCl3 in 49 mL of perchloric acid 35%), homogenized, incubated for 20 minutes and absorbant measured with spectrophotometer with a wavelength of 530 nm.

HCN Production

HCN (cyanogen) production was determined by modified method of Bakker and Schippers (1987). Exponentially grown cultures (108 cells/mL) of strains were streaked on solid agar plates supplemented with or without 4.4 g glycine/L with simultaneous addition of filter paper soaked in 0.5% picric acid in 1% Na in the upper lids of plates along with uninoculated control. The plates were sealed with parafilm and incubated at 28 ± 1 C. Development of colour from yellow to light brown, moderate brown or strong brown was examined for putative HCN production.

Siderophore Production

Siderophore production was determined on Chrome-azurol S (CAS) medium following the method of Schwyn and Neilands (1987). The 24 hrs old cultures were spotted separately on CAS medium and incubated at 28 ± 1 ^{o}C for 48–72 hrs. Formation of orange to yellow halo around the colonies conformed the production of siderophore.

Phosphate Solubilization

For qualitative analysis, phosphate solubilization ability of isolated strains was detected by culturing the bacterial strains with filter paper dipped in bacterial suspension on Pikovskaya's agar plates. These plates were then incubated at 28 ± 1C for 3-days and observed for appearance of clearing zone around the colonies.

RESULT

Beneficial effect of *B. cereus* introduction in chili plants was noticed in experiments. The

Table 1. Effect of introduction of *Bacillus cereus* strains on germination rate, plant height, total of leaves, root length, flowering time and yields on chili plants

Isolates	Germination Rate (%)	Plant Height (cm)	Total Leaves	Root Length (cm)	Flowering Time (day after planting)	Yields (g)
B. cereus strain JN233	100.00a	71.00 a	206.67 a	35.00 a	47.00 c	139.00 a
B. cereus strain C38/15	99.33 ab	67.67 b	187.33 b	34.33 a	48.33 bc	119.67 b
B. cereus strain LGR-2	99.33 ab	71.00 a	186.67 b	35.67 a	49.33 b	98.33 c
B. cereus pPRS3a	97.33 b	63.67 c	183.67 b	36.33 a	51.67 a	84.33 cd
Control	86.00 c	55.33 d	134.00 c	24.33 b	53.67 a	72.00 d

result proved that all *B. cereus* strains were able to increase growth of chili plants compared to control (Table 1). Germination rate of chili seedlings are increased up to 100% compared to control. Plant height is increased with *B. cereus* introduction compared to control. *B. cereus* JN233 had the ability to increase germination rate, Plant height, number of leaves, root length, increase flowering time (47-days after planting) and yields.

All isolates also showed suppression of disease development caused by *R. syzygii* subsp. *indonesiensis. B. cereus* pPRS3a showed a suppression of symptom appearance (29.67 days post inoculation (dpi) compared to control (21.33 dpi) and also suppressed disease severity (1.67) compared to control (3.00). B. cereus strain JN233 which has the best ability to increase growth rate also has the best ability to fully suppress disease development with no symptom appearance until last day of observation.

All Bacillus cereus strains have the ability to produce IAA with varied range from 26.65 to 32.05 ppm. Strain JN233, C38/15 and pPRS3a have the ability to produce HCN. Strain JN233 and C38/15 have the ability to produce siderophore. *B. cereus* strain JN233 is the only strain that is soluble in phosphate. Strain JN233 has the ability of all attributes tested (IAA, HCN, Siderophore, Phosphate solubilization). These attributes on strain JN233 produced confirmed its ability to promote growth rate, yields and control *R. syzygii* subsp. *indonesiensis* in chili plants.

DISCUSSIONS

Bacillus as a seed treatment has been used for biological control of soilborne phytopathogens that affect many host plants (El-Hassan and Gowen, 2006; Morsy *et al.*, 2009; Zhang *et al.*, 2009) Introduction of *Bacillus* species from the rhizosphere of different crops has widely been studied previously. Mehta *et al.* (2010) have reported the presence of almost all

Table 2. Effect of introduction of *Bacillus cereus* strains and inoculated with *R. syzygii* subsp. *indonesiensis* in symptom appearance and severity

Isolates	Symptom Appear (dpi)	Severity
B. cereus strain JN233	60.00* a	0.00 d
B. cereus strain C38/15	42.00 b	0.67 c
B. cereus strain LGR-2	43.00 ab	0.67 c
B. cereus pPRS3a	29.67 bc	1.67 b
Control	21.33 c	3.00 a

PGP attributes in *Bacillus circulans* MTCC 8983.In vitro inhibition of various phytopathogens by *B. subtilis* ME488 has also been reported (Chung *et al.*, 2008). Idris *et al.* (2007) first demonstrated the production of reasonable quantities of IAA from Gram-positive bacterium *B. amyloliquefaciens* FZB42 and IAA production was enhanced when the bacterium was fed with tryptophan. In vitro IAA production by *Bacillus* spp. in

Table 3. Plant Growth Promoting Attributes of *Bacillus ereus* strains

Isolates	IAA (ppm)	HCN	Siderophore	Phosphate Solubilization
B. cereus strain JN233	32.05	+	+	+
B. cereus strain C38/15	28.9	+	+	-
B. cereus strain LGR-2	27.1	-	-	-
B. cereus pPRS3a	26.65	+	-	-

significant amount has also been reported by Singh *et al*. (2008) and Mehta *et al.* (2010). It has been observed that the role of bacterial IAA in different plant-microbe interactions highlights the fact that bacteria use this phytohormone to interact with plants as part of their colonization strategy, including phytostimulation and circumvention of basal plant defense mechanisms (Ahmad *et al.,* 2008; Samuel and Muthukkaruppan, 2011).

The ability of several isolates to solubilize phosphate in vitro shows the possible application of the isolates in crop fields. Rodriguez and Fraga (1999) demonstrated that *Pseudomonas* and other phosphate solubilizing bacteria (PSB) like Bacillus sp. were capable of increasing the availability of phosphorus in soil. Specifically, all isolates showed their potential to be developed as inoculants for alkaline soil, based on the ability to solubilize phosphate bounded by calcium which mostly exists in alkaline soils, whereas in the acidic soil, phosphate is mostly fixed by Fe or Al (Glodstein, 1995). Our study revealed that *Bacillus cereus* strains JN233 have ability to solubilize phosphate and to increase growth rate.

Siderophores directly stimulate the biosynthesis of other antimicrobial compounds by increasing the availability of these minerals to the bacteria, would suppress the growth of pathogenic organisms *viz*., *F. oxysporum* and *R. solani,* function as stress factors in inducing host resistance (Haas and Defago, 2005; Joseph *et al.,* 2007; Wahyudi *et al.,* 2011). B. megaterium from tea rhizosphere produces siderophores which help in plant growth promotion and disease reduction (Chakraborty *et al.,* 2006). *Bacillus cereus* UW 85 produce siderophore which can be used as efficient rhizobacteria to increase the crop yield (Husen, 2003).

Bacillus spp. are considered to be the safe microorganisms that hold remarkable abilities for synthesizing a vast array beneficial substances (Stein, 2005). *Bacillus* spp. having potent growth promoting traits such as IAA production, phosphate solubilization, nitrogen fixation, and biocontrol attributes like production of HCN, siderophore, hydrolytic enzymes and antibiotics have been isolated (Senthilkumar *et al.,* 2009). *Bacillus* strains have the advantage of being able to form endospores which confers them high stability as biofungicides or biofertilizers (Schisler *et al.,* 2004).

Our experiment found that B. cereus strain JN233 have the best ability to promote growth rate, increase yields and suppress *R. syzygii* subsp. *indonesiensis*, by mechanisms of PGPR attributes by producing IAA, solubilize phosphate, producing siderophore and Cyanide acid (HCN). It could be concluded that all Bacillus cereus strains in this experiment can increase growth rate and disease suppression compared to control *B. cereus* strain JN233 have best ability to promote growth rate, increase yields and suppress *R. syzygii* subsp. *indonesiensis*. All Bacillus cereus strain has variable PGPR attributes. *B. cereus* Strain JN233 has all attributes characterized in this experiment.

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