

Indigenous endophyte bacteria ability to control *Ralstonia* and *Fusarium* wilt disease on chili pepper

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Abstract. Yanti Y, Warnita, Reflin, Busniah M. 2018. *Indigenous endophyte bacteria ability to control Ralstonia and Fusarium wilt disease on chili pepper. Biodiversitas 19: 1532-1538.* Bacterial wilt and *Fusarium* wilt caused by *Ralstonia syzigii* subsp. *indonesiensis* and *Fusarium oxysporum* f.sp. *capsici* (*Foc*), respectively are the most damaging vascular pathogens in chili pepper (*Capsicum annuum* L.) and many other crops limiting their production, worldwide. Various strategies have been developed to control wilt pathogens including the application of chemical pesticides, which generally considered as the most effective and fastest strategy for plant disease management. However, effective chemicals for wilt pathogens of chili pepper plants are not available, yet. Endophytic bacteria considered as one of options to control vascular wilt disease because of its ability to live and colonize in internal roots of plants. Previous research has been done to select endophytic indigenous bacteria isolates which can promote growth rate of chili pepper. The purpose of the research was to identify the selected indigenous endophyte bacteria isolates acquired from our previous study using 16S rRNA identifications and to screen the selected endophytic indigenous bacteria to control both *R. syzigii* subsp. *indonesiensis* and *Foc*. Results from 16S rRNA analysis showed that all of 9 isolates were identified as *Bacillus* spp., such as *Bacillus cereus* ATCC 14579, *Bacillus pseudomycoloides* strain NBRC 101232, *Bacillus toyonensis* strain BCT-7112, *Bacillus thuringiensis* strain ATCC 10792, *Bacillus weihenstephanensis* strain DSM 11821, *Bacillus mycoloides* strain 273, *Bacillus cereus* strain NBRC 15305, *Bacillus bingmayongensis* strain FJAT-13831 and *Bacillus manliponensis* strain BL4-6. Our results showed that most of endophytic bacteria isolates application could control both bacterial and *Fusarium* wilt diseases. Six out of nine isolates can suppress *R. syzigii* subsp. *indonesiensis* without developing any symptoms and five isolates could suppress symptoms of *Foc*. Isolates *Bacillus pseudomycoloides* strain NBRC 101232 *Bacillus thuringiensis* strain ATCC 10792 and *Bacillus mycoloides* strain 273 were potential for control *Foc* and *R. syzigii* subsp. *indonesiensis* in chili pepper.

Keywords: Endophyte, *Fusarium*, indigenous, *Ralstonia*

INTRODUCTION

Capsicum annuum L. is an economically important crop consumed as spicy substitution or ingredient in the world (Paul et al. 2013). Due to its high value and consumption rate, the annual trade of chilies reaches approximately 17% of total spicy ingredient trades in the world (Ahmed et al. 2000). However, chili productions can be decreased due to pathogen attack such as *Ralstonia syzigii* subsp. *indonesiensis* (formerly named *R. solanacearum* (Safni et al. 2014)) and *Fusarium oxysporum* f.sp. *capsici*, the main causal agents of wilt disease. Bacterial wilt and *Fusarium* wilt are a serious disease and a major constraint in the production of chili pepper (*Capsicum annuum* L.) and other crops in tropical, subtropical, and warm temperate regions of the world (Sahi and Khalid 2007; Ayana and Fininsa 2016). The disease has consistently caused an annual yield loss of about 10-80% (Vanitha et al. 2009). The combination of both two pathogens may increase the yield loss of chili pepper, worldwide.

Although a large number of methods had employed to control the disease (including cultural practices, crop rotation, and host resistance), the current integrated management strategies for the disease are still limited

(Pradhanang et al. 2003). Efforts to find primary factors and effective control methods to mediate the disease severity should be done (Yao and Allen 2006). On the other hand, to meet the high demand of chili peppers worldwide, the large amount of fertilizers, herbicides, and pesticides has been applied to increase productivity of chili in the last 50 years (FAO 2010). Fungicide and other pesticide usages may cause to environmental and health concerns (Aktar et al. 2009). In order to reduce pesticides usage, biological control is one of a promising strategy to be applied as part of an integrated management of pesticides usage reduction. In recent years, the focus of disease management has shifted by controlling diseases using biocontrol agents, which are safer and more promising alternative than synthetic pesticides. Biological control is one of alternative soil-borne disease management by using beneficial microorganisms (Zavaleta 2000). One of biological controls is endophyte bacteria which colonizing in the internal healthy plants, become a renewed interest due to their potential in agriculture (Strobel et al. 2004).

Endophytes are microorganisms that reside within internal tissues of living plants without any visible harming effects on the host plant (Paul et al. 2013). Bacterial

endophytes colonize in ecological niche that similar to phytopathogens, making them suitable as biocontrol agents (Berg et al. 2005). Unlike phytopathogens, endophytic bacteria do not cause visible harm to the host; but they can exert beneficial effects on the plant, including defense against pathogens and increase growth and development by producing plant growth promoting substances and/or by fixating nitrogen from the atmosphere (Glick 2012; Mercado-Blanco and Lugtenberg 2014). The internal tissues of plants provide a safer environment compared to the rhizosphere and phylloplane where the introduced bacterial population must compete for nutrients. These advantages envisage the use of endophytic bacteria for more successful biological control of plant diseases (Sturz and Christie 1998).

The proven advantages of using endophytes for controlling plant diseases or biocontrol agents are their well adapted to live inside the plants, their reliable suppression of vascular disease (Lin et al. 2013) and no cause for environmental contamination. There is some evidence that endophytes can control plant diseases (Ramesh et al. 2009). Several studies had shown that endophyte bacteria from various genera can control phytopathogens, such as *Pseudomonas fluorescens* PICF7 can suppress verticillium wilt on olive trees (Mercado-Blanco et al. 2004), *Bacillus subtilis* Lu1144 can control *Ralstonia solanacearum* on mulberry (Ji et al. 2008), *B. amyloliquefaciens* Bg-C31 can control *R. solanacearum* on chili pepper (Hu et al. 2010), and on many other plants.

Our Previous study had screened 9 best endophyte bacteria isolates, which has potential to plant growth promotion (Yanti et al. 2017). However, the ability of these strain to control pathogens is not identified, yet. This Research purposed to identify those isolates using 16S rRNA identifications and screen those selected endophytic indigenous bacteria to control both *R. syzigii* subsp. *indonesiensis* and *Fusarium oxysporum* f.sp. *capsici* (*Foc*).

MATERIALS AND METHODS

Study area

This research was done in Microbiology Laboratory, Department of Plant Protection, and screen house, Faculty of Agriculture, Universitas Andalas, Padang, Indonesia from April to October 2017. □

Endophyte bacteria isolates preparation

All isolates used in this study were collected in microtube 1.5 mL. All isolates re-cultured in Nutrient Agar (NA) media in petri-dish by striking the collection to the plates and incubated for 72 h in room temperature. The growth culture was then regrowth in the same media and incubated for 48 h. The pure colony growth in the plates then used for further study.

16S rRNA bacteria identifications

Endophyte isolates identified based on its 16S rRNA gene. Bacterial DNA extracted by following protocol of PureLink Genomic DNA Mini Kit (Invitrogen, Thermo

Scientific Inc. USA). The extracted DNA was then amplified using PCR with universal primers (27F and 1492R). PCR conditions were done by following Xiong et al. (2014) consisting of denaturation at 94°C for 1 min, annealing at 54°C for 30 s and extension at 72°C for 1 min for 30 cycles and final extension for 30 minutes. The 16S fragment then sequenced in Macrogen Inc. (Korea).

Indigenous endophyte bacteria assay to control

Ralstonia and *Fusarium* wilt

All 9 strain bacteria were used to assay their ability to control *Ralstonia syzigii* subsp. *indonesiensis* and *Fusarium oxysporum* f.sp. *capsici* (*Foc*). Research conducted in two separates experiments both for each pathogen. Research done in completely randomized designs with 3 replications with 10 treatments (9 strains of indigenous endophyte bacteria and control).

Multiplication of isolates

One pure colony of indigenous endophyte bacteria (IEB) isolates was added to 25mL of Tryptic Soy Broth (TSB, HiMedia®) in culture bottle (50mL) and was incubated in rotary shaker for 24 hours. 1 mL preculture was transferred to 150 mL of sterile coconut water in Erlenmeyer flask for main culture and was incubated for 2x24 hours (Yanti et al. 2017). Suspension of IEB isolates from main culture was diluted with comparison to McFarland scale 8 (Density estimated 10⁸ CFU/mL).

Seeding and planting

Seeding of chili pepper seed was done in seed tray. Seed introduced with IEB isolates with dipping method in IEB isolates suspension with density 10⁸ CFU/mL (Compared with McFarland Scale 8). Seeding was done in 21 days. All 21-day old chili pepper seedlings (from previous methods) were then planted to polybag containing soil and organic matters (2: 1 v/v) (Yanti et al. 2013). Seedlings dipped in the same IEB isolates suspensions for each pathogen were planted in triplications.

Pathogens treatment

Ralstonia syzigii subsp. *indonesiensis* acquired from diseased plants were isolated and cultured in TZC medium. All *R. syzigii* subsp. *indonesiensis* isolates were then assayed in chili plants (2 weeks old) to select the most virulence pathogens. *R. syzigii* subsp. *indonesiensis* was cultured in TZC agar, which was then suspended to 10⁸ CFU/mL, and inoculated for 2 weeks after planting with root wounding methods. While, *Foc* acquired from diseased plants were isolated and cultured in Potato Dextrose Agar. All *Foc* isolates were then assayed in chili seedlings (21 days after seeding) to select the most virulence pathogens. The most virulence *Foc* was then cultured in rice for 1 week, followed by inoculation with 10g inoculum for 1 week before planting.

Parameter observed

Parameter observed in this research were i.e incubation time, incidence, severity, plant height, number of leaves, and yields.

RESULTS AND DISCUSSION

16S rRNA bacteria identifications

The 16S rDNA of all the 9 IEB isolates were amplified with the primers 27F and 1492R. PCR amplicons of 16S rRNA of about 1,500 bp were observed as discrete bands in gel electrophoresis (Fig. 1). The estimated sizes of the sRNA fragments of all the amplified product were in parallel line with 1.500 bp marker, which was close to 1.465 bp, the expected amplicons size of PCR using primers 27F/1492R. The 16S rRNA sequences of endophyte bacterial isolates were compared their homology with the available sequences in the GeneBank Database using BLAST. On the database of 16S rRNA sequence, all isolates were all similar to *Bacillus* genus. Furthermore, the isolates were identified similar to various species from GenBank database, which were *Bacillus cereus* ATCC 14579 (AGBE3.3.BB), *B. pseudomycooides* strain NBRC 101232 (SLBE1.1.SN), *B. toyonensis* strain BCT-7112 (AGBE1.2.TL), *B. thuringiensis* strain ATCC 10792 (SLBE3.1.BB), *B. weihenstephanensis* strain DSM 11821 (SLBE1.1.BB), *B. mycooides* strain 273 (SLBE1.1.AP), *B. cereus* strain NBRC 15305 (SLBE3.1.AP), *B. bingmayongensis* strain FJAT-13831 (AGBE2.1.TL) and *B. manliponensis* strain BL4-6 (SLBE2.3.BB) all with varied similarity (Table 1).

Plant growth of chili pepper introduced with indigenous endophyte bacteria

In this study, we aimed to identify endophytic bacteria that had been previously identified as *Bacillus* spp., which associated with the roots of chili pepper that have potential

to promote growth rate of chili pepper plants. Chili pepper introduced with 11 indigenous endophytic bacteria had ability to promote growth shown from increased height and number leaves of plants. Indigenous endophytic isolates can increase chili pepper growth better than control (Table 2). All strains promote height varies from 71.33 cm to 108.67cm, compared to control, 66.33 cm. It means that all strains had effectivity to promote chili pepper heights (7.54 to 63.83%) compared to control. *B. pseudomycooides* strain NBRC 101232 and *B. thuringiensis* strain ATCC 10792 were the best strains to promote plant height. *B. pseudomycooides* strain NBRC 101232 applied in plants also increase the number of leaves with highest effectivity (56.02%) compared to control.

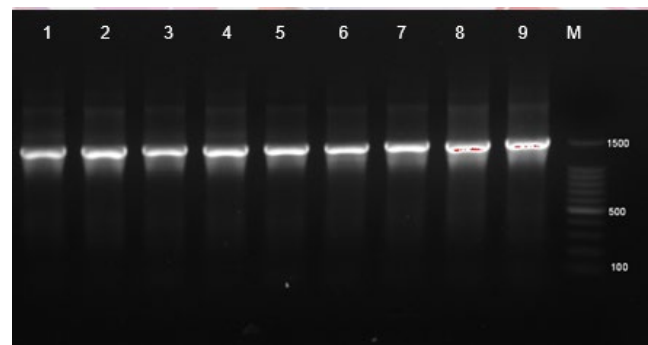


Figure 1. 16S rRNA PCR electrophoresis results of IEB isolates, 1. AGBE3.3.BB; 2=SLBE1.1.SN; 3. AGBE1.2.TL; 4. SLBE3.1.BB; 5. SLBE1.1.BB; 6. SLBE1.1.AP; 7. SLBE3.1.AP; 8. AGBE2.1.TL; 9. SLBE2.3.BB; M. Marker.

Table 1. Species similarity of indigenous endophyte isolates based on 16S rRNA identifications using BLAST-N

Isolates	16S rRNA identifications using BLAST	Similarity
AGBE3.3.BB	<i>Bacillus cereus</i> ATCC 14579	96
SLBE1.1.SN	<i>Bacillus pseudomycooides</i> strain NBRC 101232	93
AGBE1.2.TL	<i>Bacillus toyonensis</i> strain BCT-7112	92
SLBE3.1.BB	<i>Bacillus thuringiensis</i> strain ATCC 10792	94
SLBE1.1.BB	<i>Bacillus weihenstephanensis</i> strain DSM 11821	95
SLBE1.1.AP	<i>Bacillus mycooides</i> strain 273	95
SLBE3.1.AP	<i>Bacillus cereus</i> strain NBRC 15305	94
AGBE2.1.TL	<i>Bacillus bingmayongensis</i> strain FJAT-13831	94
SLBE2.3.BB	<i>Bacillus manliponensis</i> strain BL4-6	92

Table 2. Height, number of leaves and yields of chili pepper introduced with indigenous endophyte bacteria

Strains	Plant height (cm)	Effectivity	No. of leaves	Effectivity	Yields (kg)	Effectivity
<i>B. pseudomycooides</i> strain NBRC 101232	108.67 ^a	63.83	52.00 ^a	56.02	1.29 ^a	186.67
<i>B. thuringiensis</i> strain ATCC 10792	105.33 ^a	58.80	48.67 ^{bc}	52.03	1.22 ^a	171.11
<i>B. mycooides</i> strain 273	98.33 ^b	48.24	50.67 ^{ab}	46.02	0.99 ^b	120.00
<i>B. cereus</i> strain NBRC 15305	97.33 ^{bc}	46.74	46.00 ^{cd}	38.01	0.89 ^c	97.78
<i>B. bingmayongensis</i> strain FJAT-13831	93.33 ^c	40.71	44.00 ^{de}	32.01	0.84 ^{cd}	86.67
<i>B. manliponensis</i> strain BL4-6	87.00 ^d	31.16	39.33 ^f	26.01	0.79 ^d	75.56
<i>B. thuringiensis</i> strain ATCC 10792	82.33 ^d	24.12	39.33 ^f	18.00	0.64 ^e	42.22
<i>B. weihenstephanensis</i> strain DSM 11821	73.67 ^e	11.07	42.00 ^{ef}	18.00	0.53 ^f	17.78
<i>B. mycooides</i> strain 273	71.33 ^e	7.54	39.33 ^f	18.00	0.56 ^f	24.44
Control	66.33 ^f		33.33 ^g		0.45 ^g	

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

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

Strains	Disease development time	Effectivity	Disease Incidence (%)	Effectivity	Severity	Effectivity
<i>B. pseudomycooides</i> strain NBRC 101232	42.00 ^a	110.00	0.00	100.00	0.00 ^c	100.00
<i>B. thuringiensis</i> strain ATCC 10792	42.00 ^a	110.00	0.00	100.00	0.00 ^c	100.00
<i>B. mycooides</i> strain 273	42.00 ^a	110.00	0.00	100.00	0.00 ^c	100.00
<i>B. thuringiensis</i> strain ATCC 10792	42.00 ^a	110.00	0.00	100.00	0.00 ^c	100.00
<i>B. weihenstephanensis</i> strain DSM 11821	42.00 ^a	110.00	0.00	100.00	0.00 ^c	100.00
<i>B. cereus</i> strain NBRC 15305	42.00 ^a	110.00	0.00	100.00	0.00 ^c	100.00
<i>B. bingmayongensis</i> strain FJAT-13831	37.33 ^{ab}	86.65	33.33	66.67	0.67 ^{bc}	83.25
<i>B. mycooides</i> strain 273	37.33 ^{ab}	86.65	33.33	66.67	0.67 ^{bc}	83.25
<i>B. manliponensis</i> strain BL4-6	32.67 ^b	63.35	66.67	33.33	2.00 ^b	50.00
Control	20.00 ^c		100		4.00 ^a	

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

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

Strains	Disease development time	Effectivity	Disease Incidence (%)	Effectivity	Severity	Effectivity
<i>B. pseudomycooides</i> strain NBRC 101232	42.00 ^a	106.59	0.00	100.00	0.00 ^d	100.00
<i>B. thuringiensis</i> strain ATCC 10792	42.00 ^a	106.59	0.00	100.00	0.00 ^d	100.00
<i>B. mycooides</i> strain 273	42.00 ^a	106.59	0.00	100.00	0.00 ^d	100.00
<i>B. cereus</i> strain NBRC 15305	42.00 ^a	106.59	0.00	100.00	0.00 ^d	100.00
<i>B. bingmayongensis</i> strain FJAT-13831	42.00 ^a	106.59	0.00	100.00	0.00 ^d	100.00
<i>B. manliponensis</i> strain BL4-6	39.33 ^{ab}	93.46	66.67	33.33	0.67 ^{cd}	83.25
<i>B. thuringiensis</i> strain ATCC 10792	31.00 ^c	52.48	66.67	33.33	2.67 ^{ab}	33.25
<i>B. weihenstephanensis</i> strain DSM 11821	32.67 ^{bc}	60.70	100.00	0.00	2.00 ^{bc}	50.00
<i>B. mycooides</i> strain 273	30.67 ^c	50.86	66.67	33.33	2.67 ^{ab}	33.25
Control	20.33 ^d		100		4.00 ^a	

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



Figure 2. Chili plants 3 weeks after inoculated with *R. syzigii* subsp. *indonesiensis*, introduced with *B. pseudomycooides* strain NBRC 101232 (left) and control (right)



Figure 3. Chili plants 3 weeks after inoculated with *Foc*, introduced with *B. thuringiensis* strain ATCC 10792 (left) and control (right)

Besides, it had good ability to promote growth rate, all strains also had good ability to increase yields of chili pepper. All strains increase the yields with varied effectivity from 17.78 to 186.67%. Strains *B. pseudomycooides* strain NBRC 101232, *B. thuringiensis* strain ATCC 10792 and *B. mycooides* strain 273 respectively increase yields to 1.29 kg, 1.22 kg, and 0.99 kg compared to control 0.45 kg.

Introduction of *Bacillus* spp. strains showed decrease of disease development time, incidence and severity of *Ralstonia* wilt diseases (Table 3, Fig. 2). Three isolates of *Bacillus* shown decrease of incidence up to 100% and shows no symptoms until the end of observations. Introduction of *Bacillus* spp. strains also showed promoting plant growth after inoculation of pathogen. Strain *B. pseudomycooides* strain NBRC 101232, *B. thuringiensis*

strain ATCC 10792, *B. mycooides* strain 273, *B. thuringiensis* strain ATCC 10792, *B. weihenstephanensis* strain DSM 11821 and *B. cereus* strain NBRC 15305 have highest ability to promote growth rate of chili pepper and could also suppress pathogen attack with no symptoms of diseases shown.

Introduction of *Bacillus* spp. strains also could decrease disease development time, incidence and severity of diseases of *Fusarium* (Table 4). Five isolates of *Bacillus* shown decrease of *Fusarium* wilt incidence up to 100% and shows no symptoms until the end of observations. Those strains were *B. pseudomycooides* strain NBRC 101232, *B. thuringiensis* strain ATCC 10792, *B. mycooides* strain 273, *B. cereus* strain NBRC 15305 and *B. bingmayongensis* strain FJAT-13831. Strain *B. pseudomycooides* strain NBRC 101232, *B. thuringiensis* strain ATCC 10792, *B. mycooides* strain 273, *B. thuringiensis* strain ATCC 10792, *B. weihenstephanensis* strain DSM 11821 and *B. cereus* strain NBRC 15305 have highest ability to promote growth rate of chili pepper and could also suppress both *Fusarium* and *Ralstonia* wilt disease with no symptoms of diseases was shown.

Discussion

The exploration of the diversity of plant microbiome is an interesting way to acquire new effective bacteria as potential biocontrol agents. Bacteria and other microbes naturally residing within plants without causing any damage to their host can be good candidates as biocontrol agents. Several microorganisms, called endophytes, are well adapted to their host, and make balanced antagonism between plant host-endophyte and endophyte-endophyte interactions (Schulz and Boyle 2005). Our Previous 9 best endophyte bacteria isolate having potential as plant growth promotion also performed good traits to control *Fusarium* and *Ralstonia* wilt disease.

In this study, the best IEB isolates from the previous research were identified by its 16S rRNA analysis using universal 27F/1492R primers. The electrophoresis analysis results showed that all the IEB amplicons were in parallel with the expected amplicon size of 1.500bp. These results were in accordance with Webster et al. (2003) stated that 27F/1492R primers used in PCR would produce about 1.500bp length of amplicons, which close to ideal length of 16SrRNA gene used for DNA comparisons with database. The present result showed that all endophytes belonged to *Bacillus* genera using 16S rRNA analysis with BLAST. The gene sequence of the 16S rRNA facilitated the putative taxonomic identification of isolates. Recent description (Kim et al. 2012; Dourado et al. 2012) also revealed that 16S rRNA gene sequence analysis could give proper identification of bacteria. Among the species of Plant Growth Promoting Rhizobacteria (PGPR), *Bacillus* spp. had been known as powerful genera. Various of *Bacillus* species can promote the health and control diseases by plant pathogens suppressions, or by nutrients competitions like iron and phosphate or indirectly fixing nitrogen (McSpaden Gardener 2004). *Bacillus* spp. is one of the biological control agents that have shown inhibitory effects against a considerable number of plant pathogens, and the

antibiotics that it produces are generally assumed to be responsible for the control activity. *Bacillus*, or the use of their metabolites, may be an alternative or supplementary method to chemical plant protection (Mojica-Marin et al., 2008). Khalid et al. (2004) reported that species of *Bacillus* have a very high efficiency in plant root colonization and the production of phytohormones, resulting in improved crop yield.

Bacillus as biocontrol agents has been used for biological control of soilborne phytopathogens that affect many host plants (Zhang et al. 2009). Mehta et al. (2010) have reported the presence of almost all 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). Our research showed that our strains could suppress pathogenic effects of *R. syzigii* subsp. *indonesiensis* and *Fusarium oxysporum* in plants. Antagonistic mechanisms of endophytic bacteria towards pathogens are mainly related to antibiosis, competition, lysis, induction of plant defense and plant growth (Berg and Hallman, 2006). Kloepper et al., (1999) reported that induced systemic resistance (ISR) might be one of the most important operating mechanisms when it is dealing with biocontrol of systemic plant pathogens, such as bacterial wilt. There are also researches reported that bacterial inoculations could be triggered by bacterial inoculations. Ji et al., (2008) also reported that endophytic *B. subtilis* strains could induce ISR of mulberry plants against bacterial wilt disease.

Our research also found that all *Bacillus* spp. strains acquired from the previous research could both control *Ralstonia* and *Fusarium* wilt and promote growth rate of chili pepper in field condition. We conclude that these happened due to the strain ability to produce several beneficial substances that affect growth promotion in chili peppers. *Bacillus* spp. are considered as one of the safest 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 to high stability as biofungicides or biofertilizers (Schisler et al. 2004). Idris et al. (2007) firstly 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 significant amount has also been reported by Singh et al. (2008) and Mehta et al. (2010). It has been highlighted that the role of bacterial IAA in different plant-microbe interactions is 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).

Our results showed that all the endophyte strains used are effective in controlling *Ralstonia* and *Fusarium* Wilt Disease on Chili Pepper. Those strains might be used as promising indigenous biocontrol agent strains for successful management of *Ralstonia* and *Fusarium* Wilt disease, moreover, those strains also had opportunity for wider usage. However, the further study to know their role in plant growth promotion and pathogen suppression, and moreover for its commercial use should be done.

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