Potential of Entomopathogenic *Bacillus thuringiensis* as Plant Growth Promoting Rhizobacteria and Biological Control Agents for Tomato *Fusarium* Wilt

Jiaheling Qi[1], Daigo Aiuchi[2], Masayuki Tani[3], Shin-ichiro Asano[4], Masanori Koike*[5]

[1]The United Graduate School of Agricultural Sciences, Iwate University, Iwate Japan;
[1,2,3,5]Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Japan
[4]Department of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

Abstract— *Bacillus thuringiensis has been used as an effective bioinsecticide because it produces the proteins Cry and Cyt, which are highly toxic to insects in certain situations. However, recently, B. thuringiensis was used as a biological control agent that can suppress plant disease. In this study, the antagonistic activities of B. thuringiensisAS17 japonensisand AS18 kurstaki against the fungal pathogen Fusarium oxysporum f.sp. lycopersici (FOL) were examined using a dual culture technique. Furthermore, B. thuringiensis strains suppressed the development of wilt symptoms caused by FOL in tomato plants. After inoculating six strains of B. thuringiensis suspension following inoculation of FOL, the development of wilt symptoms became less than control, especially with B. thuringiensisAS17 japonensis and AS20 CR371-H. Furthermore, we proved that B. thuringiensis strains are plant growth promoting rhizobacteria (PGPR) that can promote plant growth. Seed germination and shoot elongation were promoted by treating the tomato seeds with a bacterial culture filtrate and a bacterial suspension.*

Keywords— Bacillus thuringiensis, Fusarium oxysporum f.sp. lycopersici, plant growth promoting rhizobacteria (PGPR), tomato.

I. INTRODUCTION

Tomato (*Solanum lycopersicum*) is an important vegetable crop.In tomato cultivate, it is often hampered by vascular wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* (FOL)W.C. Synder and H.N.Hans. FOL is a serious fungal pathogen of the tomato plant. The most important symptom of this pathogen is vascular wilt. FOL can cause a huge loss of tomato quantity and quality, and it has the ability to colonize the roots of a large number of fireweeds and to produce resistant spore structures, so it can exist in mostsoils (Ailton et al., 2007). This pathogen is difficult to control with traditional methods, such as preplant soil fumigation and other cultural methods, so it is a better way to choose bio control to control this disease (Archana et al., 2010).

In general, some effective means of controlling FOL include disinfection of the soil and planting material by using fungicidal chemicals, crop rotation with non-hosts of the fungus, or by using resistant cultivars (Ana et al., 1997; Benhamou et al., 1998). Since the current available control methods, such as chemical control, are either low efficient or difficult to apply, methods to control *Fusarium* wilts caused by FOL have been studied for a long time (Kotan et al., 2009). The chemical control measures destroy balances in the microbial community, which may cause the loss of abundance beneficial organisms, such as insect natural enemies, and may also lead to the evolution of resistant strains of the pathogen. Breeding resistant plant varieties can be difficult in the absence of dominant genes and development of new races of the pathogen that overcome host resistance (Jetiyanon and Kloepper, 2002). Furthermore, increasingly, there are more restrictions on utilization of fungicides due to public concern about harmful effects on the human health and environment, and residues in the food. Hence, there is a need for developing attractive management strategies that are virtually efficient and environmentally safe (Sudhamoy et al., 2009).

Bacillus thuringiensis has been used as an effective bioinsecticide (Roh et al., 2007; Schnepf et al., 1998). The specificity of*B. Thuringiensis* is showed highly beneficial in agricultural biotechnology. Unlike most insecticides, *B. thuringiensis* insecticides are highly toxic against target insects and friendly towards beneficial insects, non-target organisms such as humans and wildlife (Bravoet al., 2011). It is also not harmful to the environment. *B. thuringiensis* has been used as an alternative to chemical pesticides for decades by organic farmers to control insects. At present, *B. thuringiensis* is the only "microbial insecticide" in widespread use (Cherif et al., 2003, 2008; Dong et al., 2002).

Plant growth promoting rhizobacteria (PGPR) promote plant growth and suppress plant disease by colonizing plant roots, reducing plant pathogen populations in the soil, and maintaining a beneficial effect on plant growth. Similarly, the sporulating gram-positive bacteria such as *Bacillus* spp. have also been used successfully as potential biological control

agents (BCAs) to control plant disease (Kloepper et al., 2004). Also, some studies have reported that *Bt* was successful endophytic colonization insoybean, cotton, cabbage, rice bean and so on, even with concomitant production of Crytoxins; the efficient *Bt* colonization of cabbage seedlings roots suggests this might be in fact the main route of its penetration in the plant (Argôlo-Filho, et al., 2014). Similarly, *Bt* was able to colonize the roots of certain legumes, which resulted in an increase of nodulation and growth of the plants and *Bt* produces toxins that can reduce pests or diseases attacks (Mishra, et al., 2009). Recently, *B. thuringiensis* has also attracted great attention as a biological control agent to suppress plant diseases (Zhou, Yet al., 2008). Therefore, the new view is that the insecticide *B. thuringiensis* can be used as PGPR to control plant disease. Moreover, the activity of *B. thuringiensis* which can suppress bacterial wilt disease has been examined in tomato plants (Hyakumachi et al., 2013).

The objective of this study was to confirm whether *B. thuringiensis* could suppress the wilt disease caused by *Fusarium oxysporum* f.sp. *lycopersici* (FOL), and to verify if *B. thuringiensis* can act as plant growth promoting rhizobacteria (PGPR), which can promote plant shoot elongation and seed germination.

II. MATERIALS AND METHODS

2.1 Fungal pathogen preparation

Fusarium oxysporum f.sp. *lycopersici* (FOL) race 2 was used in the pathogenicity experiments. The isolate (FOL) was grown on potato dextrose agar (PDA) medium at 26°C for 7 days in a culture dish and then stored at 5°C. The isolate, recovered as needed from storage, was grown on PDA at 26°C for 7 days prior to inoculation.

2.2 Bacterial strains and inoculum preparation

The tested *B. thuringiensis* strains *(AS15 sotto, AS16 israelensis, AS17 japonensis, AS18 kurstaki, AS19 roskildiensis, AS20 CR371-H)* used for the present investigation were obtained from Research Faculty of Agriculture, Applied Bioscience Applied Molecular Biology Laboratory, Hokkaido University. For inoculum preparation, six strains of *B. thuringiensis* were inoculated in Luria Bertanibroth (LB) and grown for 1 week at 30°C. For the preparation of *B. thuringiensis* culture filtrate (CF), the six strains of bacteria were inoculated in liquid LB broth and grown for 24 h with constant shaking (150 rpm) at 28 ± 2°C. The culture obtained at the stationary phase was centrifuged at 6000 rpm for 10 min and the bacterial cells were resuspended in sodium phosphate buffer (100 mM; pH 7.0). The bacterial concentration was adjusted to 3×10^8cfu/ml.

2.3 Antagonistic activity of *B. thuringiensis*

The antifungal activity of six strains of *B. thuringiensis* against *FOL* were evaluated by dual culture method on PDA, in triplicate. FOL ($9mm^2$ conlony) was inoculated in the middle of a PDA plate, and at the same time, the six strains of *B. thuringiensis* were inoculated at the edge of the PDA plate separately. A PDA plate that was only inoculated with the pathogen was treated as a control. The inoculated PDA plates were incubated at 28 ± 2°C. When the control PDA plates were full of mycelium, the length of a clear zone caused by *B. thuringiensis*, between the *B. thuringiensis* colony and *FOL* colony were observed.

2.4 Pot experiment

Subsequently, evaluation of the suppressive effect on *FOL* by six strains of *B. thuringiensis* in the soil condition (pot experiment) was conducted. Bacterial culture filtrate and bacterial suspension were prepared. Tomato seedlings were grown at 25°C. The roots of 4-week-old tomato seedlings were treated with bacterial culture at 3 ml/pot (3×10^8cfu/ml). FOL-infested soil was prepared. Sterile soil and maize flour were mixed completely at the rate of 2:1 (v/v) to make the culture soil. In a flask filled with 12ml distilled water, 40 ml of culture soil was taken, and sterilized in an autoclave. The FOL was inoculated in the sterilized culture soil, and stored in a 25°Cincubator for 7 days. Sterile soil (1 L) and 5% (v/v) FOL infested soil were mixed completely and put in a sterilized plastic box to make the FOL infested soil. The bacteria-treated tomato seedlings were placed in the FOL-infested soil (4 pots/box). Tomato seedlings treated with distilled water alone were used as a control. Three weeks after pathogen inoculation, the fresh weight, shoot length, and root length were measured and the wilting score was evaluated. Wilting score was evaluated based on leaf symptoms of wilting as follows: 0 = no wilt symptoms; 1 = <25% of wilting leaves; 2 = 26–50% of wilting leaves; 3 = 51–75% of wilting leaves; 4 = 76–100% of wilting leaves(Bora et al., 2004).

2.5 The activity of Bt strains on promotion of tomato seed germination

To evaluate the PGPR effects of *B. thuringiensis* culture filtrate on seed germination, we performed bacterial treatment of tomato seeds. Bacterial culture filtrate and bacterial suspension were prepared. Seeds of tomato were surface sterilized with 1% sodium hypochlorite for 1 min and washed with sterilized water 3 times, then immersed in bacterial culture filtrate and

bacterial suspension. After 24 h, the bacterial culture filtrate (or bacterial suspension) was thrown away and the seeds were dried for 12 h in sterile Petri dishes. Tomato seeds soaked in phosphate buffer alone were used as a control treatment. Bacterial culture filtrate (or bacterial suspension) treated tomato seeds were placed at 25°C (10 seeds/dish). After 3 days, the seed germinations were evaluated after inoculation. Using same seed treatment method, pot experiments were carried out in a completely randomized design in a greenhouse at 25°C with five replicates. Bacteria treated seeds were sown in individual 9-cm-diameter pots containing sterilized soil (Shanmugam et al., 2011). For the pot experiments, plant fresh weight, dry weight, and height were measured 4 weeks after inoculation.

2.6 Root and shoot elongation

Plant growth-promoting activity was evaluated by the standard roll towel method (ISTA, 1993). Bacterial culture filtrate and bacterial suspension were prepared. Seeds of tomato were surface sterilized with 1% sodium hypochlorite for 1 min and washed with sterilized water 3 times, then immersed in bacterial culture filtrate and bacterial suspension. After 24 h, the bacterial culture filtrate (or bacterial suspension) was thrown away and the seeds were dried for 12 h in sterile Petri dishes (diameter = 90 mm). Tomato seeds soaked in phosphate buffer alone were used as a control treatment. Bacteria - treated seeds were placed between folds of wet germination paper. A roll towel method involved rolling of the seeds in wet germination paper. First, a label with details regarding sample, number of replicate seeds and date was placed on the top left hand corner. Next, a wet paper towel was placed over the label. The seeds were then placed 2 cm below the edge of the sheet in a row using forceps. A strip of paper was also wetted and placed over the seeds to keep them moist and to prevent them from being displaced while rolling. The wet paper towel was then rolled, using a rubber band to prevent unfurling of the roll. The rolled paper was subsequently placed in water at 25°C. After 2 weeks the shoot and root elongation were measured using a ruler with fine-scale measurements.

2.7 Data analysis

Plant fresh and dry weight, plant root and shoot length, wilting score, seed germination rate, plant length, shoot and root elongation were analyzed using analysis of variance (ANOVA) in SSRI for Windows 7®. The significance of differences within treatments was separated by the least significant difference test at 5%.

III. RESULTS

3.1 Antagonistic activity of *B. thuringiensis*

In the dual culture assays for antifungal activity, six strains of *B. thuringiensis* were tested. As a result *B. thuringiensis*, *AS17 japonensis*, and *AS18 kurstaki* inhibited the mycelial growth of the pathogen FOL significantly, with an inhibition clear zone. These results show that *B. thuringiensis AS17 japonensis and AS18 kurstaki* have antagonistic activity against FOL, which can inhibit the mycelial growth of FOL (Fig. 1).

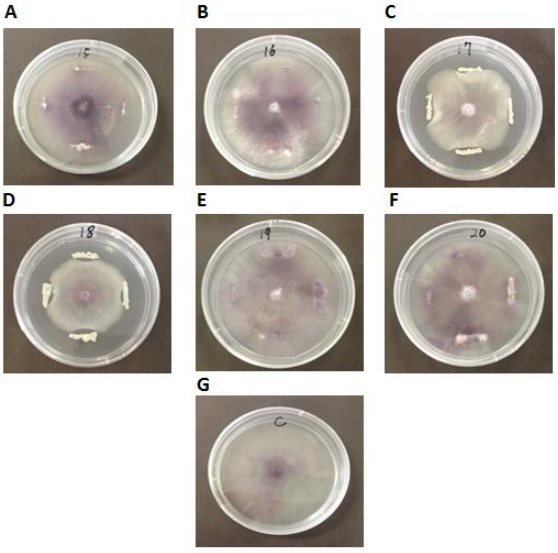

FIG. 1. ANTAGONISTIC BACTERIA SELECTION WAS EVALUATED BY DUAL CULTURE TECHNIQUE ON PDA. PDA PLATE THAT WAS ONLY INOCULATED WITH THE FOL WAS TREATED AS A CONTROL. THE CLEAR ZONE CAUSED BY *AS17 JAPONENSIS* AND *AS18 KURSTAKI* AGAINST FOL WERE CLEARLY SHOWN IN THIS PICTURE (FIG. 1C, D).
A: *AS15 SOTTO TREAMENT*; B: *AS16 ISRAELENSIS TREAMENT*; C: *AS17 JAPONENSIS TREAMENT*; D: *AS18 KURSTAKI TREAMENT*; E: *AS19 ROSKILDIENSIS TREATMENT*; F: *AS20 CR371-H TREATMENT*; G: *CONTROL*.

3.2 Pot experiment

The results showed that tomato roots treated with *B. thuringiensis* culture filtrate suppressed the growth of FOL and the development of wilt symptoms to less than the pathogen control; the strains *B. thuringiensis, AS17 japonensis and AS20 CR371-H* had a significant effect (ANOVA, F = 11.64; df = 7, 16; P<0.01) (Fig. 2). A highly antagonistic activity of *B. thuringiensis* strain, *AS17 japonensis* was assessed with and without inoculation with FOL and compared with untreated controls in a pot. The bacterial treatment led to a significant reduction in the wilting score, from 83.3% to 25% (Fig. 2), with less disease incidence compared to pathogen treatment alone. The bacterial treatments recorded a significant increase (ANOVA, F=13.12; df=1, 41; P< 0.01) in tomato plant weight from 11.2% to 220.1%(Table 1) relative to the pathogen control, and increased the tomato shoot length from 43.1% to 108% (Table 1) relative to the pathogen control, and root length from 13.7% to 65.6% (Table 1) relative to the pathogen control.

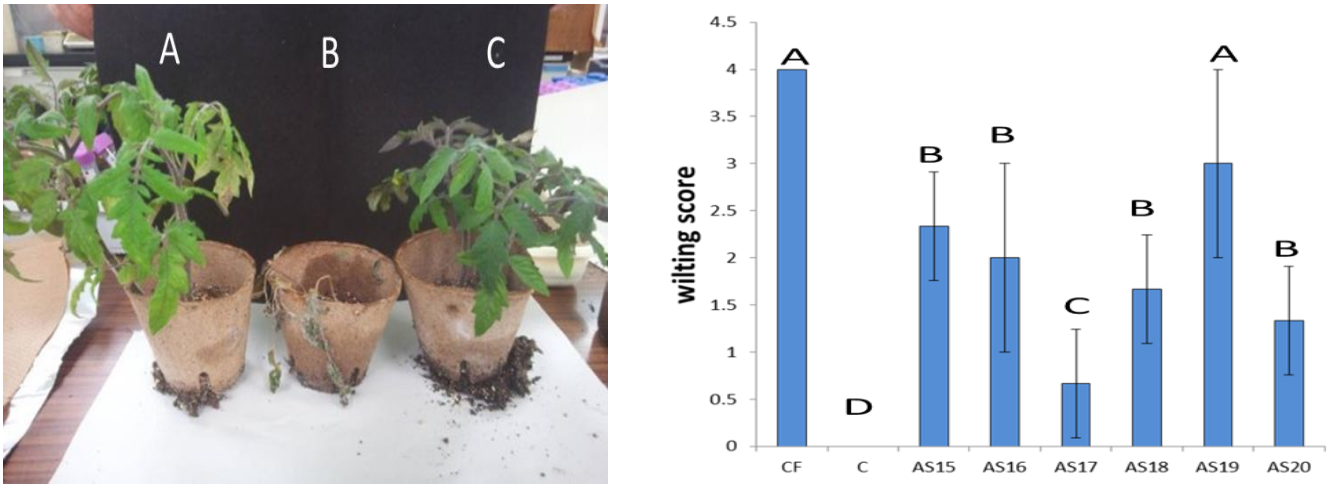

FIG. 2. DEVELOPMENT OF *FUSARIUM* SYMPTOMS IN TOMATO PLANTS BY TREATING THEIR ROOTS WITH *B. THURINGIENSIS* STRAINS. (A) TOMATO SEEDLINGS TREATED WITH *AS17 JAPONENSIS* AND FOL; (B) TOMATO SEEDLINGS TREATED WITH *FUSARIUM OXYSPORUM F.SP. LYCOPERSICI RACE 2* ALONE; (C) TOMATO SEEDLINGS TREATED WITH DISTILLED WATER ALONE. WILTING SCORE OF TOMATO IN POT EXPERIMENT. WILTING SCORE IN TOMATO PLANTS WITH THEIR ROOTS TREATED WITH THE SUSPENSION OF *B. THURINGIENSIS*. CF: PATHOGEN CONTROL; C: UNTREATED CONTROL.
Mean and standard deviation of three replicates per experiment are presented. Values followed by different letters were not significantly different according to the Tukey test at a 0.05 level of confidence.

TABLE 1
THE SHOOT, ROOT LENGTH AND FRESH WEIGHT OF THE BACTERIA AND PATHOGEN TREATED TOMATO SEEDLINGS IN POT EXPERIMENT.

	PC	C	AS15	AS16	AS17	AS18	AS19	AS20
shoot length, cm	26.00 ± 12.70c	79.00 ± 12.30a	48.40 ± 11.10b	48.40 ± 13.80b	54.10 ± 8.30ab	46.30 ± 13.80b	37.20 ± 21.10b	51.20 ± 17.30b
Root Length, cm	10.90 ± 5.08c	26.00 ± 2.16a	9.80 ± 3.20c	18.10 ± 7.24b	12.40 ± 3.14c	15.80 ± 3.50b	9.70 ± 1.86c	16.60 ± 8.29b
fresh weight, g	5.78 ± 1.83B	36.55 ± 12.37A	6.44 ± 5.41B	9.47 ± 4.97B	8.73 ± 5.93B	7.98 ± 2.41B	5.68 ± 4.23B	18.50 ± 10.47A

PC: pathogen control; C: untreated control.
Mean and standard deviation of three replicates per experiment are presented. Values followed by different letters were significantly different according to the Tukey test at a 0.05 level of confidence.

3.3 The effect of *Bt* strains on promotion of tomato seed germination

All the six strains of *B. thuringiensis* increased the germination rate of tomato seeds compared to untreated control tomato seeds (Fig. 3), with increases ranging from 35.7% to 107.1% (Fig. 3). Amongst all the strains, *AS18 kurstaki* showed the highest germination rate. In the pot experiment, *B. thuringiensis* strains promoted plant length from 4% to 28.5% versus untreated control (Table. 2), promoted plant fresh weight from 3.8% to 108.4% versus untreated control (Table. 2), promote plant dry weight from 35.3% to 201.9% versus untreated control (Table. 2).

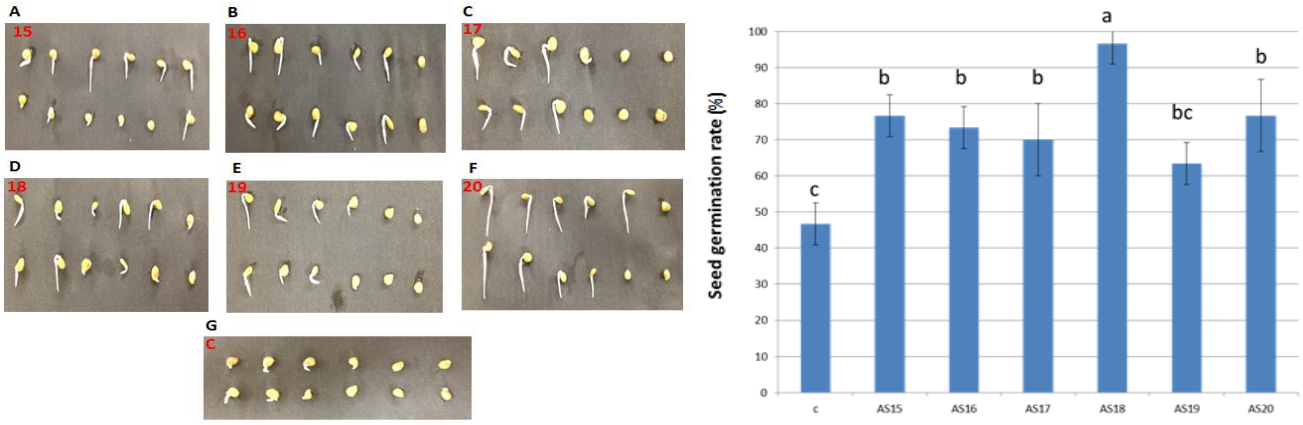

FIG. 3. THE ACTIVITY OF BT STRAINS ON PROMOTION OF TOMATO SEED GERMINATION WAS DONE BY SEED TREATMENT. C: UNTREATED CONTROL. MEAN AND STANDARD DEVIATION OF THREE REPLICATES PER EXPERIMENT ARE PRESENTED. VALUES FOLLOWED BY DIFFERENT LETTERS WERE SIGNIFICANTLY DIFFERENT ACCORDING TO THE TUKEY TEST AT A 0.05 LEVEL OF CONFIDENCE.
A: *AS15 SOTTOTREAMENT*; B: *AS16 ISRAELENSIS TREAMENT;* C: AS17 *JAPONENSIS TREAMENT;* D: *AS18 KURSTAKI TREAMENT;* E: *AS19 ROSKILDIENSIS TREATMENT;* F: *AS20 CR371-H TREATMENT*; G: CONTROL.

TABLE 2
SHOOT LENGTH, FRESH WEIGHT AND DRY WEIGHT OF BACTERIA TREATED TOMATO SEEDS IN PGPR POT EXPERIMENT.

	C	AS15	AS16	AS17	AS18	AS19	AS20
length, cm	24.47 ± 4.72a	27.50 ± 4.60a	31.45 ± 2.27a	28.33 ± 3.06a	27.23 ± 3.85a	25.45 ± 3.66a	31.25 ± 2.56a
Fresh weight, g	1.95 ± 1.17b	3.18 ± 1.02b	4.06 ± 1.51a	3.24 ± 1.01b	2.80 ± 1.05b	2.03 ± 0.83b	4.03 ± 0.99a
Dry weight, g	0.05 ± 0.04b	0.13 ± 0.05b	0.15 ± 0.07a	0.11 ± 0.04b	0.11 ± 0.03b	0.07 ± 0.02b	0.15 ± 0.02a

C: untreated control. Mean and standard deviation of three replicates per experiment are presented. Values followed by different letters were not significantly different according to the Tukey test at a 0.05 level of confidence.

3.4 The activity of *Bt* strains on tomato root and shoot elongation

The results of the roll towel experiment showed that all of the *B. thuringiensis* strains promoted tomato shoot and root elongation compared to the untreated control (Fig. 4), ranging from 87.7 to 381.1% (Fig. 4). In particular, *B. thuringiensis AS17 japonensis* promoted shoot and root elongation significantly compared to other strains and untreated controls (Fig. 4).

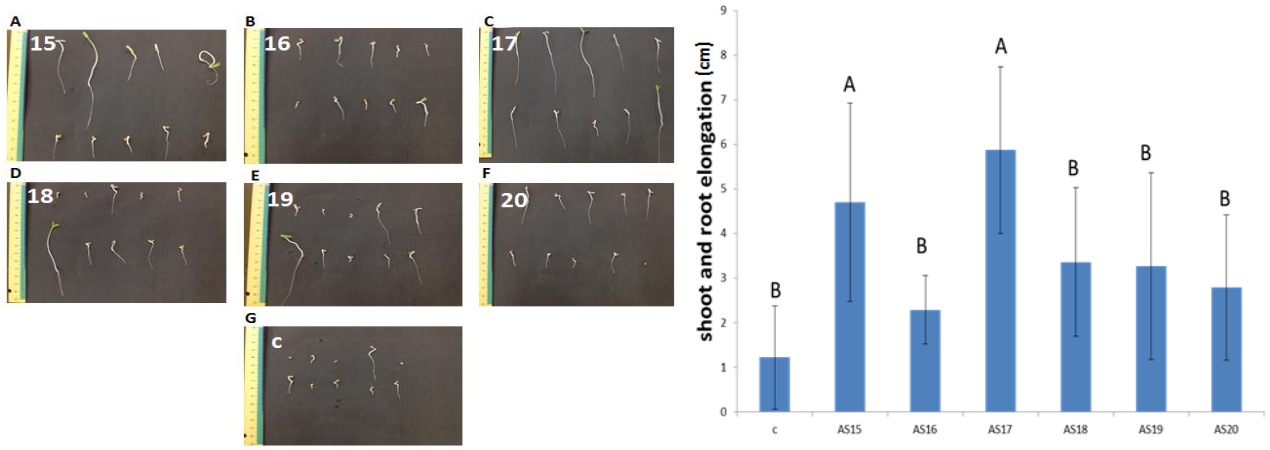

FIG. 4. SHOOT AND ROOT ELONGATION BY ROLL TOWEL METHOD. C: UNTREATED CONTROL. MEAN AND STANDARD DEVIATION OF THREE REPLICATES PER EXPERIMENT ARE PRESENTED. VALUES FOLLOWED BY DIFFERENT LETTERS WERE SIGNIFICANTLY DIFFERENT ACCORDING TO THE TUKEY TEST AT A 0.01 LEVEL OF CONFIDENCE.

A: *AS15 SOTTO TREAMENT;* B: *AS16 ISRAELENSIS TREAMENT;* C: *AS17 JAPONENSIS TREAMENT;* D: *AS18 KURSTAKI TREAMENT;* E: *AS19 ROSKILDIENSIS TREATMENT;* F: *AS20 CR371-H TREATMENT;* G: *CONTROL*.

IV. DISCUSSION

Bacillus thuringiensis has been used as an effective bioinsecticide because it produces the proteins Cry and Cyt, which are highly toxic to insects in certain situations (Betz et al., 2000). In recent years, many new functions of *Bt* have been discovered that protect plants from pathogen infection. Recently *B. thuringiensis* was used as a biological control agent that can suppress plant disease. In this study, the antagonistic activities of *B. thuringiensis AS17 japonensis* and *AS18 kurstaki* against *Fusarium oxysporum* f.sp. *lycopersici race 2* (FOL) were examined by dual culture technique. It was confirmed that *B. thuringiensis* strains can suppress the development of wilt symptoms caused by FOL in tomato plants. In addition, this study proved that *B. thuringiensis* strains were plant growth promoting rhizobacteria (PGPR) which promoted plant growth, seed germination, and shoot elongation.

Compare to chemical control, biocontrol by use of PGPR shows a potentially attractive and efficient disease management approach. PGPR are soil bacteria that able to colonize the root system of plants, and enhance plant growth and development under field conditions (Kloepper et al., 1988; Raupach et al., 1998). Indeed, PGPR are able to promote plant growth and reduce disease in crops (Jetiyanon and Kloepper2002).Since Gram-positive have the ability to form desiccation-and heat-resistant spores, it can be formulated into stable products readily (Emmert et al., 1999). Root colonizing *Bacillus* spp. is well-known by disease control and enhancement of plant growth (Kloepper et al., 2006). For example, *Bacillus subtilis* is the best-characterized member of Gram-positive bacteria, and has become a paradigm organism (Kunst et al., 1997). *B. subtilis*is treated as an excellent biocontrol agent, because it has many characteristics, such as the promotion of plant growth, formation of viable spores, production of structurally diverse antibiotics, and a ubiquitous presence in soil (Ryu et al., 2004; Bais et al., 2004; Cenci et al., 2006; Liu et al., 2006).

In this study, six strains of *B. thuringiensis (AS15 sotto, AS16 israelensis, AS17 japonensis, AS18 kurstaki, AS19 roskildiensis, and AS20 CR371-H)* were tested. All six strains of *B. thuringiensis* could suppress the growth of FOL and the development of wilt symptoms in tomato plants (Fig. 2). By treating the tomato roots with *B. thuringiensis* suspension, the wilt disease caused by FOL was significantly suppressed. But the suppressive activity might not be caused by the competition for space, nutrients and ecological niches between *B. thuringiensis* and FOL. *B.thuringiensis* generally produces several compounds, such as antimicrobial substances that include b-exotoxins, antibiotics, degrading enzymes, bacteriocins, and a signal molecule in the bacterial quorum-sensing system (Cherif et al., 2003; Dong et al., 2002). *B.thuringiensis* (Bt) strains were screened for their antibacterial, anti-insect and lactonases, chitinases, β-1,3-glucanases, and zwittermicin A (Arora et al., 2003; Cherif et al., 2003; Stabb et al., 1994). Six tested strains had at least two major insecticidal toxins genes. Concerning fungal biocontrol, all the strains inhibited the growth of *Fusariumoxysporum* and *Aspergillusflavus* and four strains had all or most of the antifungal determinants examined, with strain Bt HD932 showing the widest antifungal activity

spectrum (Raddadi et al., 2009). *B. thuringiensis* could produce compounds that occur outside of the cell, such as b-exotoxins and the antibiotic zwittermicin A (Zhou et al., 2008). The six strains of *B. thuringiensis* that were used in the experiments in this study might produce such compounds.

In all of the tested *B. thuringiensis* strains, *AS17 japonensis* and *AS18 kurstaki* showed antagonistic activity against FOL by dual culture assays (Fig. 1) and inhibited the symptoms of wilt disease in pot (Fig. 2). From these results, we conclude that *B. thuringiensis*can product antimicrobial substances against the growth of FOL. However, in the pot experiment *AS20 CR371-H* also inhibited wilt symptoms with a low wilting score and high plant weight (Table. 1), in contrast to the dual culture assays, where fungal growth was not inhibited by *AS20 CR371-H*. Therefore, there is less likelihood that antimicrobial substances were produced by *AS20 CR371-H,* unlike *AS17 japonensis* and *AS18 kurstaki* that might directly suppress the growth and spread of FOL in tomato. It was inferred that *AS20 CR371-H* suppressed the wilt disease in an indirect way. Moreover, all of the tested *B. thuringiensis* strains could promote seed germination (Fig. 3) and root and shoot elongation (Fig. 4) significantly. From this point, the mechanism of antagonistic to fungal plant pathogen and induced resistance should be clear.

PGPR enhance plant growth either directly or indirectly (Glick, 1995); direct mechanisms of plant growth promotion by PGPR can be demonstrated deficient plant pathogens or other rhizo-microorganisms and promoting the uptake of nutrients from the environment, while indirect mechanisms of plant growth promotion involve the ability of PGPR to lessen the deleterious effects of phytopathogenic organisms on crop yield (Ramamoorthy et al., 2001). PGPR strains can control plant pathogens through several mechanisms, including production of antimicrobial compounds, induced systemic resistance (ISR), and competition for spaces and nutrients with pathogens (Kloepper et al., 2004; Munees et al., 2014; Van et al., 1998). Tomato roots treated with a cell-free filtrate of *B. thuringiensis* suppressed the development of wilt symptoms caused by bacterial wilt disease *Ralstoniasolanacearum*, through the plant defense system (Jetiyanon et al., 2003; Kloepper et al., 2004). The co-activation of ET-dependent signaling pathway with the SA-dependent signaling pathway and suppression of JA-dependent signaling may play key roles in induced resistance of *B. thuringiensis* to *R. solanacearum* in tomato (Hyakumachi et al., 2014). In our data, it is also possible that some kind of elicitor compounds existed in *AS20 CR371-H*, because of non-antagonistic fungal activity and *Fusarium* wilt suppression effects.

Since PGPR are well known for their disease reduction and growth promotion abilities, biocontrol by use of PGPR shows a potentially efficient alternative disease management approach (Van, 2007). This study provides evidence for the first time that *B.thuringiensis* can be used as PGPR, which have bioactivity against a plant pathogen by suppressing the wilt disease and promoting plant seed germination, root and shoot elongation. However, why *B.thuringiensis* can promote seed germination and root and shoot elongation were not yet clear. The mechanism behind *Bacillus thuringiensis AS20 CR371-H* causing induced systemic resistance should be further studied. To further understand the mechanism of *B. thuringiensis* -induced resistance to *Fusarium oxysporum*, it will be important to identify the specific substances present in the CF that can induce resistance to *Fusarium oxysporum* in tomato. Interestingly volatile compounds and lipopeptidesee produced by *Bacillus* spp. have been identified as elicitors in ISR (Ryu et al. 2004). Therefore, characterization of the substances able to induce disease resistance will provide new insights for further evaluation of the practicality of *B.thuringiensis* as an effective biocontrol agent.

REFERENCES

[1] Archana, G., Alok, R.R., Sudhir, U.M., Dongre, A.B., 2010. Isolation, evaluation and characterization of *Bacillus subtilis* from cotton rhizospheric soil with biocontrol activity against *Fusarium oxysporum*. World Journal of Microbiology and Biotechnology 26, 1187–1194.

[2] Arora, N., Ahmad, T., Rajagopal, R.,Bhatnagar, R.K.,2003. A constitutively expressed 36 kDaexochitinase from *Bacillus thuringiensis HD1*. Biochemical and Biophysical Research Communications307, 620–625.

[3] Argôlo-Filho, R., Loguercio, L., 2014. *Bacillus thuringiensis*Is an Environmental Pathogen and Host-Specificity Has Developed as an Adaptation to Human-Generated Ecological Niches. Insects 5, 62-91.

[4] Ana, H., Blanca, L., Rafael M.J-D., 1997. Influence of chickpea genotype and *Bacillus* spp. on protection from *Fusarium* wilt by seed treatment with nonpathogenic *Fusarium oxysporum.* European Journal of Plant Pathology 103, 631–642.

[5] Ailton, R., Leonardo, S.B., 2007. Outbreak of *Fusariumoxysporum*f.sp.*lycopersici*race 3 in commercial fresh-market tomato fields in Rio de Janeiro State, Brazil. HorticulturaBrasileira 25.3, 451-454.

[6] Benhamou, N., Kloepper, J.W., Tuzun, S., 1998. Induction of resistance against *Fusarium* wilt of tomato by combination of chitosan with an endophytic bacterial strain: ultrastructure and cytochemistry of the host response. Planta 204, 153–168.

[7] Bora, T., Ozaktan, H., Gore, E., Aslan, E., 2004. Biological control of *Fusarium oxysporum* f.sp.*melonis* by wet table powder formulations of the two strains of *Pseudomonas putida*. Phytopathology 152, 471–475.

[8] Bais, H., Fall,R., Vianco, JM., 2004.Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis* roots by *Pseudomonas syringae* is facilitated by biofilm formation and surface in production. Plant Physiology 134, 307–319.

[9] Bravo, A., Likitvivatanavong, S., Gill, S., Soberón, M., 2011. *Bacillus thuringiensis*: a story of a successful bioinsecticide. Insect Biochemistry and Molecular Biology41, 423-431.

[10] Betz, F.S., Hammond, B.G., Fuchs, R.L., 2000. Safety and Advantages of *Bacillus thuringiensis*-Protected Plants to Control Insect Pests. Biological Control 32, 156–173.

[11] Cherif, A., Chehimi, S., Limem, F., Hansen, B.M., Hendriksen, N.B., Daffonchio, D., Boudabous, A., 2003. Purification and characterization of the novel bacteriocinentomocine 9, and safety evaluation of its producer, *Bacillus thuringiensis* subsp. entomocidus HD9. Journal of Applied Microbiology 95, 990–1000.

[12] Cherif, A., Rezgui, W., Raddadi, N., Daffonchio, D., Boudabous, A., 2008. Characterization and partial purification of entomocin 110, a newly identified bacteriocin from *Bacillus thuringiensis*subsp. *entomocidus* HD110. Microbiological Research 163, 684–692.

[13] Cenci, G., Trotta, F., Caldini, G., 2006. Tolerance to challenges miming gastrointestinal transit by spores and vegetative cells of *Bacillus clausii*. Journal of Applied Microbiology 101, 1208–1215.

[14] Dong, Y., Zhang, Q., Xu, J.L., 2002. Identification of quorum-quenching N-acylhomoserinelactonases from *Bacillus* species. Applied and Environmental Microbiology68, 1754–1759.

[15] Emmert, E., Handelsman, J., 1999. Biocontrol of plant disease: a (Gram) positive perspective. FEMS Microbiology Letters 171, 1–9.

[16] Glick, B.R., 1995. The enhancement of plant growth by free-living bacteria. Canadian Journal of Microbiology 41,109–117.

[17] Hyakumachi,M., Nishimura,M., Arakawa,T., Asano, S.,Yoshida,S, Tsushima,S., Takahashi,H., 2013. *Bacillus thuringiensis* suppresses bacterial wilt disease caused by *Ralstoniasolanacearum* with systemic induction of defense-related gene expression in tomato. Microbes and Environments 28 (1), 128–134.

[18] ISTA, 1993. Proceedings of international seed test association, international rules for seed testing. Seed Science and Technology 21, 1–152.

[19] Jetiyanon, K., Kloepper, J.W., 2002. Mixtures of plant growth promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. Biocontrol 24, 285–291.

[20] Jetiyanon, K., Fowler, W.D., Kloepper, J.W., 2003. Broad spectrum protection against several pathogens by PGPR mixtures under field conditions in Thailand. Plant Disease 87, 1390–1394.

[21] Kloepper, J.W., Ryu, C.M., Zhang, S., 2004. Induced systemic resistance and promotion of plant growth by Bacillus spp. Phytopathology 94, 1259–1266.

[22] Kunst, F., Ogasawara, N., Moszer, I., Albertini, A., Alloni, G., 1997. The complete genome sequence of the Grampositive bacterium *Bacillus subtilis*. Nature 390(6657), 249–256.

[23] Kloepper, J.W., Hume, D.J., Scher, F.M., et al., 1988. Plant growth promoting rhizobacteria on canola (rapeseed). Plant Disease 72, 42–46.

[24] Kloepper, J.W., Ryu, C.M., 2006. Bacterial endophytes as elicitors of induced systemic resistance. In: Schulz, B., Boyle, C., Siebern, T. (Eds.), Microbial Root Endophytes. Springer-Verlag, Heildelberg, 33–51.

[25] Kotan, R., Sahin, F.b., Demirci, E., Eken, C., 2009. Biological control of the potato dry rot caused by *Fusarium* species using PGPR strains. Biological Control 50, 194–198.

[26] Munees, A., Mulugeta, K., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saud University–Science 26, 1–20.

[27] Mishra, P.K., Mishra, S.,Selvakumar, G.,Bisht, J.K.,Kundu, S., Gupta, H.S., 2009. Coinoculation of *Bacillus thuringiensis*-KR1 with *Rhizobium.leguminosarum*enhances plant growth andnodulation of pea (*Pisum. sativum*L.) and lentil (*Lens culinaris*L.). World Journal of Microbiology and Biotechnology25, 753–761.

[28] NRaupach, G.S., Kloepper, J.W., 1998. Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88, 1158–1164.

[29] Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V., Samiyappan, R., 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Protection 20, 1–11.

[30] Raddadi, N., Belaouis, A., Tamagnini, I., Hansen, B.M., Hendriksen, N.B., Boudabous A., Cherif, A., Daffonchio, D., 2009. Characterization of polyvalent and safe *Bacillus thuringiensis* strains with potential use for biocontrol. Journal of Basic Microbiology 49, 293–303.

[31] Roh, J.Y., Choi, J., Li, M.S., Jin, B.R., Je, Y.H., 2007. *Bacillus thuringiensis* as a specific, safe, and effective tool for insert pest control. Journal of Microbiology and Biotechnology 17, 547–559.

[32] Ryu, C.M., Murphy, J.F., Mysore, K.S., Kloepper, J.W., 2004. Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against *Cucumber mosaic virus* by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. The Plant Journal 39, 381–392.

[33] Schnepf, E., Crickmore, N., van Rie L.D., Baum, J., Feitelson, J., Zeigler, D.R., Dean, D.H., 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews 62, 775–806.

[34] Shanmugam, V., Kanoujia, N., 2011. Biological management of vascular wilt of tomato caused by *Fusarium oxysporum* f.sp.*lycopersici* by plant growth-promoting rhizobacterial mixture. Biological Control 57, 85–93.

[35] Stabb, E.V., Jacobson, L.M. and Handelsman, J.,1994. Zwittermycin A producing strains of *Bacilluscereus*from diverse soils. Applied and Environmental Microbiology60, 4404–4412.

[36] Sudhamoy, M., Nirupama, M., Adinpunya, M., 2009. Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato. Plant Physiology and Biochemistry 4, 642–649.

[37] Van Loon, L.C., 2007. Plant responses of plant growth-promoting rhizobacteria. European Journal of Plant Pathology 119, 243–254.

[38] Van Loon, L.C., Bakker, P.A.H.M., Pieterse, C.M.J., 1998. Systemic resistance induced by rhizosphere bacteria. Annual Review of Phytopathology 36, 453–483.

[39] Zhou, Y., Choi, Y.L., Sun, M., Yu, Z., 2008. Novel roles of *Bacillus thuringiensis* to control plant diseases. Applied Microbiology and Biotechnology 80, 563–572.