Pantoea agglomerans SWg2 colonizes mulberry tissues, promotes disease protection and seedling growth

Authors: Jie Xie, Ping Shu, Gary Strobel, Jie Chen, Junhong Wei, Zhonghuai Xiang, and Zeyang Zhou

Xie, Jie, Ping Shu, Gary Strobel, Jie Chen, Junhong Wei, Zhonghuai Xiang, and Zeyang Zhou. "Pantoea agglomerans SWg2 colonizes mulberry tissues, promotes disease protection and seedling growth." Biological Control 113 (October 2017): 9-17. DOI: 10.1016/j.biocontrol.2017.06.010.

Pantoea agglomerans SWg2 colonizes mulberry tissues, promotes disease protection and seedling growth

Jie Xie[a,1], Ping Shu[a,1], Gary Strobel[b], Jie Chen[a], Junhong Wei[a], Zhonghuai Xiang[a], Zeyang Zhou[a,c,*]

[a] *Key Laboratory of Sericultural Biology and Genetic Breeding, Ministry of Agriculture, College of Biotechnology, Southwest University, Chongqing 400715, China*
[b] *Department of Plant Sciences, Montana State University, Bozeman, MT 59717, USA*
[c] *College of Life Science, Chongqing Normal University, Chongqing 400047, China*

A B S T R A C T

Mulberry bacterial blight caused by *Pseudomonas syringae* pv. *mori*, is one of the most serious diseases on mulberry (*Morus alba* L.), and threatens sericulture development. An endophytic *Pantoea agglomerans* SWg2 (referred to as SWg2), originally isolated from roots of healthy mulberry, is extremely inhibitory activity to *P. syringae*. Greenhouse tests were first conducted to examine the biological control properties of SWg2. A suspension of SWg2, introduced to the host plant, significantly reduced disease symptoms caused by *P. syringae*. Likewise, when a suspension of SWg2 was sprayed onto plantlets 2 days before inoculation with *P. syringae*, its disease control efficacy was up to 65.6% even after inoculation with *P. syringae* 18 days later. Interestingly, the control effect of the traditional agrochemical streptomycin was just 33.2% at 18 days post inoculation (DPI), although it was 55.6% at 9 DPI. Furthermore, SWg2 promoted the growth of mulberry seedlings. The immersion of 10^7 CFU mL^{-1} suspension of SWg2 had the most distinctive growth-promoting role since the seed germination rate and radicle length development were enhanced up to 100% and 144.2%, respectively. Based on the green fluorescent protein (GFP) labeling technique and a re-isolation method, it was determined that the SWg2 strain colonized and spread in mulberry seedlings. The bacteria entered seedlings through the areas of emergence of root hairs and cracks in the rhizodermis. Eventually these cells spread to other tissues of the plant with colonization occurring mainly in the intercellular spaces. Furthermore, the GFP-tagged SWg2 strain could be detected in stems and leaves, but the quantity was smaller than that in roots. There appears to be no outward negative effects of SWg2 on treated plants and silkworms feed on them in a normal manner. This work indicates that *P. agglomerans* SWg2 possesses the biological potential to provide protection of its host against the pathogen of mulberry bacterial blight will at the same time promoting the growth and vigor of the host plant.

1. Introduction

Mulberry (*Morus alba* L.), the unique food source of the silkworm (*Bombyx mori*), but it has the potential of being infected by a plethora of diseases throughout the year in subtropical and temperate zones, which results in a severe threat to the production and quality of mulberry leaves (Kumar and Gupta, 2004). Mulberry bacterial blight alias mulberry rotten head disease, caused by *Pseudomonas syringae* pv. *Mori* (Boyer and Lambert) (Young et al., 1978), is one of the most common and serious diseases of mulberry. There are a myriad of symptoms associated with this disease including crinkle leaf, black blight, and broken leafstalks. The disease spreads rapidly and can cause major damage (Kuai, 2012). Furthermore, feeding silkworm larvae with infected leaves is detrimental to the development of the cocoon and negatively affects the normal characteristics of the worms. Meanwhile, improper application of chemical pesticides to suppress this disease may also result in hazards to the silkworms. In addition, the method of disease control using agrochemicals has disadvantages in environmental contamination and the ultimate development of resistance by the pathogens. Therefore, there is a requirement to find an alternative management style that is both safe to silkworms and friendly to the environment. Biological control against plant pathogens by plant treatment with antagonistic pseudomonads was firstly reported by Lam in PNAS (Lam et al., 1987). Since then, it has been a promising strategy

for plant protection from the damage caused by pathogens, and has been practiced worldwide (Erdogan and Benlioglu, 2010; Kloeppe et al., 1999; Luo et al., 2010; Mizumoto et al., 2007).

Plant endophytic bacteria and fungi, which reside within plant tissues mainly in intercellular (rarely in intracellular) spaces without showing any external disease symptoms (Ryan et al., 2008), have been widespread employed as the biological control agents. Antagonistic endophytic bacteria and fungi can inhibit the pathogen through the following mechanisms: production antibiotics, competition for nutrients and niches, induction of systemic resistance of the host plant and plant-growth-promotion. The above factors can contribute to the suppression of the invasion of phytopathogens (Calderón et al., 2014).

This strain SWg2 of *P. agglomerans* (GenBank, KC783460) is an antagonistic endophytic bacterium against *P. syringae* pv. *mori*, isolated from healthy mulberry roots (Zhang et al., 2013). *Pantoea agglomerans* also known as *Erwinia herbicola* (Löhnis) Dye are members of the *E. herbicola-Enterobacter agglomerans* cluster belonging to the *Enterobacteriaceae*, which are ubiquitous in nature, inhabiting soil, water, plants, animals and humans (Graham and Hodgkiss, 1967; Gavini et al., 1989). As endophytic bacteria, isolates of the *P. agglomerans* are reported to have the abilities of producing diverse antibiotics like Herbicolin I (APV), Pantocin A (Herbicolin O), Pantocin B, Andrimd and AGA (alanylgriseoluteic acid) (Pidot et al., 2014; Sammer et al., 2009), along with stimulating growth properties of several crop plants (Feng et al., 2006; Quecine et al., 2012). However, no attempt has been made to protect mulberry plants from the blight disease using endophytic *P. agglomerans* as a plant colonization agent. This work was aimed to investigate endophytic *P. agglomerans* SWg2 as a potential agent for the biocontrol mulberry bacterial blight. In addition the growth promoting ability of this organism as well as its plant colonization potential was studied, and finally preliminary studies were made on how it may promote the plant growth.

2. Materials and methods

2.1. Strains and plasmid

SWg2 strain was isolated from the surface-disinfected root of a healthy mulberry sampled from the mulberry experimental field of Southwest University, Chongqing, China (29 °48′47″ N, 106 °24′30″ E) (Zhang et al., 2013). This strain possessed significant antagonistic activity against *Pseudomonas syringae* pv. *mori* by the inhibitory halo assay in an agar plate. And then, it was identified to be *Pantoea agglomerans* by morphological features, physiological and biochemical characteristics and molecular methods (Zhang et al., 2013). It has been stored at −80 °C in 15% glycerol. The SWg2 strain was inoculated on potato dextrose agar (PDA) medium at 28 °C and incubated 24 h before used. *P. syringae* pv. *mori*, pathogen of mulberry bacterial blight, was kindly donated by professor Wenbing Wang of the Institute of Life Science, Jiangsu University. The pathogen was grown on PDA at 28 °C and only freshly transferred cultures were used in the bioassay tests.

In order to transform *P. agglomerans* SWg2 and obtain the *gfp*-tagged strain, plasmid pGFP4412, an *Escherichia coli–Bacillus cereus* shuttle vector that contains one copy of the *gfp* (mut3a) gene controlled by the rpsD promoter of *Bacillus subtilis*, ampicillin and kanamycin resistance marker in tandem, was kindly donated by professor Xianling Ji of College of Forestry, Shandong Agricultural University.

2.2. Pot experiments assayed the biocontrol efficiency of SWg2 towards mulberry bacterial blight

The mulberry seedlings being used in the present study were free of contamination because they were derived by using nodal explants tissue culture, planted individually in 10 × 10 cm diameter pots and were cultivated in a greenhouse at natural temperatures ranging from 25 to 30 °C and a 90% relative humidity until 4–6 leaves had developed.

Fresh *P. agglomerans* SWg2 was inoculated into potato dextrose broth (PDB) and incubated at 28 °C with a shaking speed of 180 rpm for 4 days. The *P. agglomerans* SWg2 cultures were centrifuged at 12,000 rpm for 20 min, and then the cell-free supernatant was obtained by being passed through 0.22 μm micropore filter. On the other hand, after this strain was incubated in PDB liquid medium for 24–32 h, the cultures were centrifuged and the bacterial cells pellets were re-suspended in sterile distilled water which was adjusted to 7.6×10^8 colony-forming units (CFU) per milliliter to prepare the SWg2 bacterial suspension. The artificial inoculation of the mulberry bacterial blight pathogen, *P. syringae*, was made as follows: after *P. syringae* was grown in PDB liquid medium at 28 °C for 24–36 h, the cultures were adjusted to a 1×10^8 CFU mL^{-1}. The seedlings were inoculated with the suspension of *P. syringae* by wiping on the leaf with a surface-sterilized brush.

The pot experiments were designed with eleven treatments as follows: (1) the seedlings in this treatment were sprayed with sterile distilled water only as blank control; (2) **CK**, mulberry plantlets under this treatment were inoculated only with pathogen *P. syringae* and considered as a positive control; (3) **Stre −2 d**, the streptomycin solution (100 mg L^{-1}) was sprayed on plantlets 2 days prior to inoculation with *P. syringae*; (4) **Stre 0 d**, the streptomycin was applied at the same time of inoculation with *P. syringae*; (5) **Stre 2 d**, the mulberry plantlets were treated with streptomycin 2 days after inoculation with the pathogen; (6) **Supernatant −2 d**, the mulberry plantlets were applied with a cell-free supernatant of SWg2 strain 2 days before inoculation with *P. syringae*; (7) **Supernatant 0 d**, the plantlets were sprayed with SWg2 supernatant at the same time of inoculation with the pathogen; (8) **Supernatant 2 d**, the plantlets were treated with a cell-free supernatant of SWg2 strain 2 days after inoculation with *P. syringae*; (9) **Suspension −2 d**, the SWg2 whole cell suspension was sprayed onto plantlets 2 days before inoculation with *P. syringae*; (10) **Suspension 0 d**, the application of SWg2 whole cell suspension on plantlets was done at the same time of inoculation with *P. syringae*; and (11) **Suspension 2 d**, the whole cell bacterial suspension was applied 2 days after inoculation with *P. syringae*. Each treatment was replicated in three blocks with 10 pots in each and one pot with one seedling. The pots experiments were conducted in a greenhouse under natural conditions with the temperature ranging from 25 °C to 30 °C.

Generally, *Pseudomonas syringae* pv. Mori when inoculated on mulberry results in the abundant production of symptoms at about the 10th to 12th day. Therefore, the staged development of mulberry bacterial blight disease on inoculated plantlets was scored at 9 days after inoculation with *P. syringae* and verified after 18 days. The blight disease development was rated according to the scale as following: 0 (whole seedling was healthy); 1 (just several disease spots on leaves); 2 (< 25% of leaf area damaged); 3 (26–50% of leaf area damaged); 4 (51–75% of leaf area damaged); 5 (75% above of leaf area damaged or emergence of rotten head). The disease severity was quantified with percent disease index before analysis of variance. The disease indexes were calculated as the following formula (Tian and Li, 1996), and the control efficacies were calculated based on the mean disease index of each treatment (Chen et al., 2014).

$$\text{Disease index} = \frac{\sum (\text{Rating} \times \text{Number of leaves rated})}{\text{Total number of leaves} \times \text{Hingest rating}} \times 100\%$$

$$\text{Control efficacy} = \frac{\text{Mean disease index of CK} - \text{Mean disease index of the treatment}}{\text{Mean disease index of CK}} \times 100\%$$

2.3. Effect of SWg2 on mulberry seeds germination and growth of seedlings

Mulberry seeds were soaked in the SWg2 cell suspensions and prepared as described above while PDB medium instead by the King's B medium (Glickmann and Dessaux, 1995), and the suspensions were adjusted to 10^8, 10^7, 10^6 and 10^5 CFU mL^{-1} with sterilized distilled water. The cell-free supernatants of SWg2 in King's B medium were diluted in sterilized distilled water as 0-fold, 100-fold and 500-fold dilution.

Seed germination tests were done on healthy mulberry seeds by washing them in running tap water for 5 min, and then they were surface-sterilized for 1–2 min by using 75% ethanol, shaken in benzalkonium bromide (1:24) for 2 min, and followed by washing 5 times with sterile distilled water. Seeds were immersed in the SWg2 cell suspensions of different bacterial densities or the SWg2's cell-free supernatants of different dilution degree for 24 h at 25 °C with the shaking speed of 160 rpm. The seeds were placed on sterile moist filter paper and incubated at 26 °C for 8 days in Petri dishes with 12 h of light. At the fourth day of cultivation, the germination energy was calculated as the percentage of germinated seeds. The germination rate, plumule length and radicle length of seedlings were recorded at the eighth day. Surface-disinfected seeds treated in King's B medium or sterile distilled water served as a control. Each treatment was replicated three times with 20 seeds apiece.

2.4. Construction of GFP-tagged SWg2

The pGFP4412 plasmid containing the GFP gene was introduced into the wild type strain SWg2 by electroporation as described previously (Lanna Filho et al., 2013) with some modifications: Cells for electroporation were initially grown in 5 mL LB broth medium for 14–16 h at 28 °C. And then, 0.5 mL cultural suspension was added to 50 mL 2 × TY LB liquid medium and incubated at 28 °C with shaking until the OD600 reached 0.4–0.6. The cells were harvested by centrifugation at 4 °C and 4000 rpm for 15 min. After they were washed two times by cold ultrapure water, the supernatant was discarded and these cells were re-suspended in 1 mL of 10% glycerol. For electroporation, 50 μL the competent SWg2 cells was mixed with 1 μL (50 ng) of pGFP4412 plasmid in an ice-cold electroporation cuvette (2 mm) with incubation for 10 min, and then was electroporated using a Gene-Pulser (Bio-Rad) set at 2.5 kV, 25 μF, 200 Ω. The transformed cells were immediately added to 1 mL SOC medium and shaken for 1 h at 37 °C, and then plated on the LB medium containing 50 μg mL^{-1} kanamycin and 100 μg mL^{-1} ampicillin. Transformants that emitted green fluorescence stably under fluorescence microscopy (Olympus BX53, Japan) were obtained after subculturing 20 generations without selective pressure, and were designated SWg2-*gfp*.

2.5. Microscopy observation on colonization of mulberry plantlets by SWg2-gfp

Mulberry seeds were surface disinfected as described above. After being washed five times by sterilized distilled water, the seeds were placed on Petri plates with sterile water-wetted filter paper to germinate. The germinated seedlings were aseptically transplanted into the autoclaved soil, and maintained in growth chamber at 26 °C, humidity 90% and a 12 h photoperiod. When the seedlings grew to 4 leaves-old, their roots were immersed in physiological saline containing 10^8 CFU mL^{-1} SWg2-*gfp* bacteria for 5 h (Meng et al., 2014), and then they were re-planted into the soil after inoculation. The control plantlets were treated in the same way using sterile physiological saline. The roots were removed from soil at 2, 3, 4, 5, 7, and 9 days after inoculation and surfaced sterilized as described above, and cut into small pieces to mount on microscopic slides. To examine the colonization of the SWg2-*gfp* in tissues, these slides were observed under fluorescence microscopy (Olympus BX53, Japan) and a confocal laser scanning microscopy (CLSM, Olympus FV1200, Japan) with an excitation wavelength of 488 nm, and the emitted light in the range of 500–520 nm was collected for GFP examination. Images were obtained using OLYMPUS FV10-ASW 4.0 software.

2.6. Enumeration of GFP-tagged SWg2 in mulberry seedlings by re-isolation

The inoculated seedlings were surface disinfected and separated into roots, stems and leaves at 1, 3, 7, 10, 15, 21 and 28 days post inoculation. The aseptic samples were weighed and then ground in 1 mL of sterile distilled water. The homogenates were serially diluted and plated on LB medium supplemented with kanamycin (50 μg mL^{-1}) and ampicillin (100 μg mL^{-1}) to incubate 24–48 h at 28 °C, and then the CFU was counted. For each kind of tissue, the average value of triplicate plates of a specific dilution was recorded.

2.7. The effect of SWg2 on silkworms

In order to lay a good foundation for the use of the SWg2 strain for the biological control of mulberry pathogens in the field, the safety of *P. agglomerans* SWg2 was studied through using infected mulberry leaves to feed the silkworm in the normal manner. Freshly transferred SWg2 was inoculated into PDB media and incubated at 28 °C with a shaking speed of 180 rpm for 18–24 h. The SWg2 cultures were centrifuged at 8000 rpm for 5 min, and then discarded the supernatant. The SWg2 suspension was prepared through re-suspend the bacterial cells in the sterile water of equal volume, and the concentration of the bacteria was 3.5×10^7 CFU mL^{-1}. Then the suspension of SWg2 was sprayed on to the surface of the mulberry leaves in the amount of 1 mL suspension per each leaf. Meanwhile, the mulberry leaves, which were sprayed sterile water, were used as control. After the surface of mulberry leaves dried naturally, they were applied to feed healthy silkworm larvae (HL strain) at the first day and the second day of the 4th instar silkworm, fed them three times each day. And then, all silkworms were fed with clean mulberry leaves until pupation. Each treatment was 33 silkworms and the growth status of silkworm was observed every day.

2.8. Evaluation of the plant growth-promoting traits displayed by SWg2

In order to explore the growth promoting effects of SWg2, we assayed the organism for its nitrogen fixation, phosphate solubilization and indole-3-acetic acid (IAA) production activities. Nitrogen-free Ashby medium (Meng et al., 2014) was used to detect nitrogenase activity of SWg2. The Ashby plates were spot-inoculated with SWg2 and incubated at 28 °C for 7 days. The *nif*H gene was detected by PCR amplification from genomic DNA isolated from pure SWg2 bacterial colonies as described previously (Loiret et al., 2004). The primers used for PCR were *nif*H-F (5′-AAA GGY GGW ATC GGY AAR TCC ACC AC-3′) and *nif*H-R (5′-TTG TTS GCS GCR TAC ATS GCC ATC AT-3′) (Rösch et al., 2002). SWg2 strain was evaluated for potential phosphate solubilization using spot-inoculated on Pikovskaya's (PVK) agar medium containing tricalcium phosphate ($Ca_3[PO_4]_2$), the development of a hydrolysis halo indicated positive result. Phosphate solubilization activity was determined by the method described as Pratibha et al. (2007) at 456 nm with a spectrophotometer. Indole-3-acetic acid (IAA) production was estimated by inoculating this strain into King's B medium with or without 0.2 g L^{-1} L-tryptophan incubated under 28 °C. A quantitative determination of IAA-producing activity was performed as Patten and Glick (2002) examined at 530 nm using Salkowski's reagent. The determination of nitrogen fixation, phosphate solubilization and indole-3-acetic acid (IAA) production by this organism were replicated three times for each individual treatment.

2.9. Statistical analysis

Data for biocontrol assay were analyzed by Duncan's analysis of

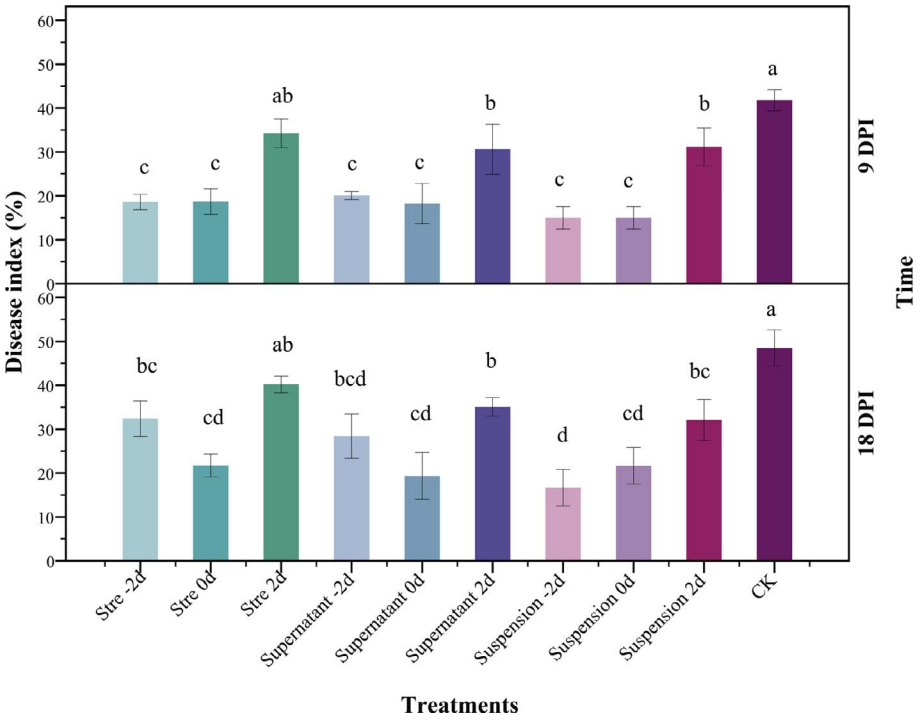

Fig. 1. Disease index of mulberry seedlings of different treatments. *Note:* DPI indicates days post inoculation. Error bars represent standard error of the mean (SE) and different letters mean significant difference at 0.05 level with Duncan's multiple range test.

variance (ANOVA) and means were compared by Duncan's test at $P \leq 0.05$. Data for bacterial promoting plant growth were evaluated by Student's *t*-test. The program SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

3. Results

3.1. Biocontrol efficacy of P. agglomerans SWg2 against mulberry bacterial blight in the greenhouse

No disease symptoms were detected in the sterile distilled water-treated blank control during the entire pot experiment, but the most serious blight symptoms were noted in the positive control (CK) in which the mulberry seedlings were inoculated only with the pathogen *Pseudomonas syringae* pv. *mori*, which showed the highest disease indexes 41.8% (9 DPI) and 48.5% (18 DPI) (Fig. 1). At 9 days post inoculation with *P. syringae* pv. *mori*, the average disease indexes of the treatment blocks of streptomycin (100 mg L^{-1}, i.e. Stre) 2 d, cell-free supernatant of SWg2 (i.e. Supernatant) 2 d and cell suspension of SWg2 (i.e. Suspension) 2 d were reduced 7.5%, 11.2% and 10.6% respectively, and that of the treatment Stre 2 d was not found significantly different from that of the positive control treatment (Fig. 1). However, the application of Stre, Supernatant and Suspension reduced the disease indexes of seedlings over 21% (from 21.8% to 26.8%) when they were applied 2 days before or simultaneously with the blight pathogen, and those significantly ($P < 0.05$) different from that of the positive control treatment (Fig. 1). Although there was no statistical significant difference in disease index between these six treatment blocks, the Suspension treatment blocks (Suspension −2 d and Suspension 0 d) exhibited the relatively lowest one, thus their control efficacies were detected as the highest one of 64.1%, and the control efficacies of the Stre treatment blocks (Stre −2 d/0 d) and Supernatant blocks (Supernatant −2 d/0 d) both were about 55.0% (Table 1). Overall, application time of the treatment was vital to the control efficacy, thus the treatment applied 2 days before or simultaneously with the blight pathogen was better than that applied 2 days after inoculation with the pathogen (Table 1).

At 18 days after inoculation with *P. syringae* pv. *mori*, there was a slight increase in the disease indices in all treatment blocks compared with those of 9 days after inoculation, especially which the Stre −2 d treatment that grew most significantly (Fig. 1), thus its control efficacy decreased distinctly from 55.6% to 33.2% (Table 1). However, the application of the suspension of SWg2 before challenge with *P. syringae* (Suspension −2 d) maintained a high level of plant protection since its control efficacy was still as high as 65.6% (Table 1). Furthermore, the application of SWg2 (Suspension or Supernatant) had relatively better control efficacy than streptomycin (Table 1).

Table 1
Control efficacies of the endophyte SWg2 against mulberry bacterial blight disease of seedlings in greenhouse.

Investigation date	Treatment time	Control efficacy (%)		
		Stre	SWg2 cell-free supernatant	SWg2 suspension
9 DPI	−2 d	55.6	52.0	64.1
	0 d	55.3	56.4	64.1
	2 d	18.0	26.8	25.5
18 DPI	−2 d	33.2	41.4	65.6
	0 d	55.2	60.2	55.3
	2 d	17.1	27.7	33.9

3.2. Growth-promoting traits of SWg2 strain on mulberry seedlings

An added benefit of using the SWg2 as a plant inoculant is its ability, as a cell suspension as well as a cell-free supernatant, to significantly ($P < 0.01$) increase the germination rate and germination energy along with the subsequent development of the seeds as compared with the sterile water treatment (Table 2). Seedling development, as measured by plumule length and radicle length, were significantly ($P < 0.01$) increased over the sterile water control with SWg2 treatments (Table 2).

Seeds soaked in SWg2 suspensions of different concentrations, were observed for germination energy as well as germination rate. The most striking effect was observed only in the treatment with a SWg2 suspension having 10^7 CFU mL^{-1}. It resulted in an increase germination energy from 70.0% to 81.7%, and promoted the germination rate from

Table 2
Impaction of the endophyte SWg2 to mulberry seeds' germination and growth.

Treatments	Dilution time	Germination energy (%)	Germination rate (%)	Plumule length (mm)	Radicle length (mm)
Sterile water	–	15.0 ± 5.0	51.7 ± 7.6	3.9 ± 0.8	5.1 ± 1.5
King's B medium	–	70.0 ± 17.3	86.7 ± 7.6	6.5 ± 1.2	11.3 ± 2.1
SWg2 suspension (10^8 CFU mL^{-1})	0	60.0 ± 13.2	90.0 ± 5.0	9.3 ± 1.9**	18.9 ± 3.2**
	10	81.7 ± 2.9	100.0 ± 0.0*	10.1 ± 2.2**	27.6 ± 3.4**
	100	75.0 ± 13.2	85.0 ± 15.0	7.8 ± 1.7	18.0 ± 3.1**
	1000	66.7 ± 2.9	81.7 ± 2.9	6.2 ± 1.2	15.1 ± 2.8**
Swg2 cell-free supernatant	0	53.3 ± 11.5	73.3 ± 12.6	10.0 ± 1.9**	26.8 ± 4.0**
	100	56.7 ± 2.9	76.7 ± 2.9	8.4 ± 2.1*	17.5 ± 3.9**
	500	78.3 ± 5.8	88.3 ± 7.6	9.8 ± 1.8**	20.2 ± 5.1**

Note: Values are the results of mean plus standard deviation, and $^{*}P < 0.05$, $^{**}P < 0.01$, *t*-Test.

86.7% to 100% as compared with the medium-immersed control (CK) (Table 2). The SWg2 suspension of 10^7 CFU mL^{-1} also extremely ($P < 0.01$) facilitated the growth of germinated seedlings, since the plumule length and radicle length of these seedlings increased by 55.4% (from 6.5 mm to 10.1 mm) and 144.2% (from 11.3 mm to 27.6 mm), respectively, as compared with CK (Table 2). Except this concentration, the other density-inoculation tended to show significant ($P < 0.01$) increase in radicle length of the seedlings (Table 2).

Likewise, immersion treatment of seeds in cell-free supernatants of different concentrations, seemly did not stimulate the germination of seeds but significantly ($P < 0.05$) increased the plumule length and radicle length of these seedlings (Table 2). Also noted, with the non-diluted cell-free supernatant, was the most significant ($P < 0.01$) growth promotion function, resulting in an increase in plumule length by 53.8% (from 6.5 mm to 10.0 mm), and a promotion in radicle length by 137.2% (from 11.3 mm to 26.8 mm), respectively, when compared to the medium control (Table 2).

3.3. Colonization and population dynamics of SWg2-gfp in mulberry seedlings

To examine the localization of SWg2 on/in mulberry seedlings, the pGFP4412 plasmid containing the *gfp* gene (*gfpmut3a*) was transferred into wild type strain by electroporation resulting in the construction of the GFP-labeled SWg2-*gfp*. The SWg2-*gfp* strain was easily characterized by its green fluorescence under a fluorescence microscope, and had a similar growth rate, the same culture phenotype and equally inhibitory activity on the pathogen as the wild type, indicating the introduced plasmid did not interfere with the normal growth and metabolism of SWg2 strain (data not be shown). The hand-cut sections of mulberry seedlings were detected by using fluorescence microscopy and confocal laser scanning microscopy at different times after inoculation with SWg2-*gfp*. The microscopic image revealed that the control using 0.85% saline water solution did not exhibit the existence of GFP-tagged bacterial cells (Fig. 2A), but there were a few SWg2-*gfp* cells attached onto the root surface of mulberry seedling inoculated with GFP-tagged bacteria of 10^8 CFU mL^{-1} just at 2 DPI (Fig. 2B). At the same time, many GFP-tagged bacterial cells were also observed to adhere along the root hair and accumulate in microcolonies at the sites of root hair emergence (Fig. 2C). And at 3 DPI, the rhizodermis showed a dense colonization pattern that large numbers of the bacteria were found as microcolonies to colonize the cracks on rhizodermis besides the sites of root hair emergence, also a few bacterial cells were observed to live in the intercellular spaces of epidermis or exodermis (Fig. 2D). Moreover some bacteria progressed towards the cortex of the root simultaneously. The bacteria congregated in the cellular spaces of exodermis and were sparsely occurring in cortical parenchyma at 4 DPI (Fig. 2E). At five days post inoculation, bacteria at the inner root were mostly distributed on cortical cells and in their intercellular spaces mainly in divergence (Fig. 2F, G). At 7 DPI, the tagged bacteria were found in cortex and inner cortex (Fig. 2H, I), and finally they were also discovered in xylem vessels and pith cells in the central cylinder at 9 DPI (Fig. 2J, K).

The enumeration of SWg2-*gfp* in roots, stems, and leaves tissues was recorded by re-isolation and quantification. The amount of bacteria colonized in root gradually decreased after an initial increase, which the population once increased to approximately 10^8 CFU g^{-1} of fresh root tissue at 3 DPI, then declined slowly to 10^3 CFU g^{-1} root at 28 DPI (Fig. 3). The GFP-tagged bacteria were observed in stems and leaves at 7 DPI. The number of bacteria fluctuated within stem was similar to that in leaf and that both changed gradually from the highest approximately 10^7 CFU g^{-1} in stems and 10^6 CFU g^{-1} in leaves to $10^{1.8}$ CFU g^{-1} in stems tissue and $10^{0.8}$ CFU g^{-1} in leaves at 28 DPI (Fig. 3). It was possible to re-isolate SWg2-*gfp* from the inoculated plant tissues. These tissues showed no visible disease damage indicating that the SWg2 was a true endophyte spreading from roots to other parts of mulberry seedlings.

3.4. The effects of SWg2 strain on the growth of silkworm

31 out of 33 experimental silkworms lived, and of these at least 26 of them pupated in the *P. agglomerans* SWg2 treatment group (Fig. 4A). The survival rate and the pupation rate were 93.9% and 78.8% respectively in this treatment. Meanwhile, in the sterile water group, 24 among 33 experimental silkworms lived and 20 of them pupated finally (Fig. 4B). The survival rate and the pupation rate were 72.7% and 60.6% respectively in this group. Overall, the survival rate and pupation rate of the *P. agglomerans* SWg2 treatment group were obviously higher than the control group. Furthermore, almost all dead silkworms died at the fifth instar stage, the body of the dead silkworm was black and soft. It thus appeared from this experiment that SWg2 was not harmful to the silkworm.

3.5. Preliminary exploration of the growth promotion mechanisms of SWg2 strain

The research results of growth-promotion mechanism of SWg2 on the mulberry seedling showed that the SWg2 strain could promote the seedling growth through the dissolution of tricalcium phosphate and IAA production, but the strain could not fix nitrogen. The SWg2 strain was able to grow and form a halo of phosphate solubilization on PVK plate (Fig. 5A). The quantitative determination of soluble phosphate showed that the concentration of soluble phosphate in the PVK liquid medium increased gradually but the pH declined during cultivation time, and the highest amount of soluble phosphate was 118.3 μg mL^{-1} after cultivation for 8 days (Fig. 5B). The development of a pink color indicated the production of IAA after addition Salkowski reagent to the culture filtrate. The IAA synthesis by SWg2 strain was significantly enhanced in the presence of L-tryptophan that the IAA production increased from 6.6 to 11.7 μg mL^{-1} at the eighth day within the culture medium supplemented with this amino acid (Fig. 6). SWg2 was unable to grow on a nitrogen-free medium plate, and the *nif*H gene was unable to duplicate through PCR amplification with its genomic DNA.

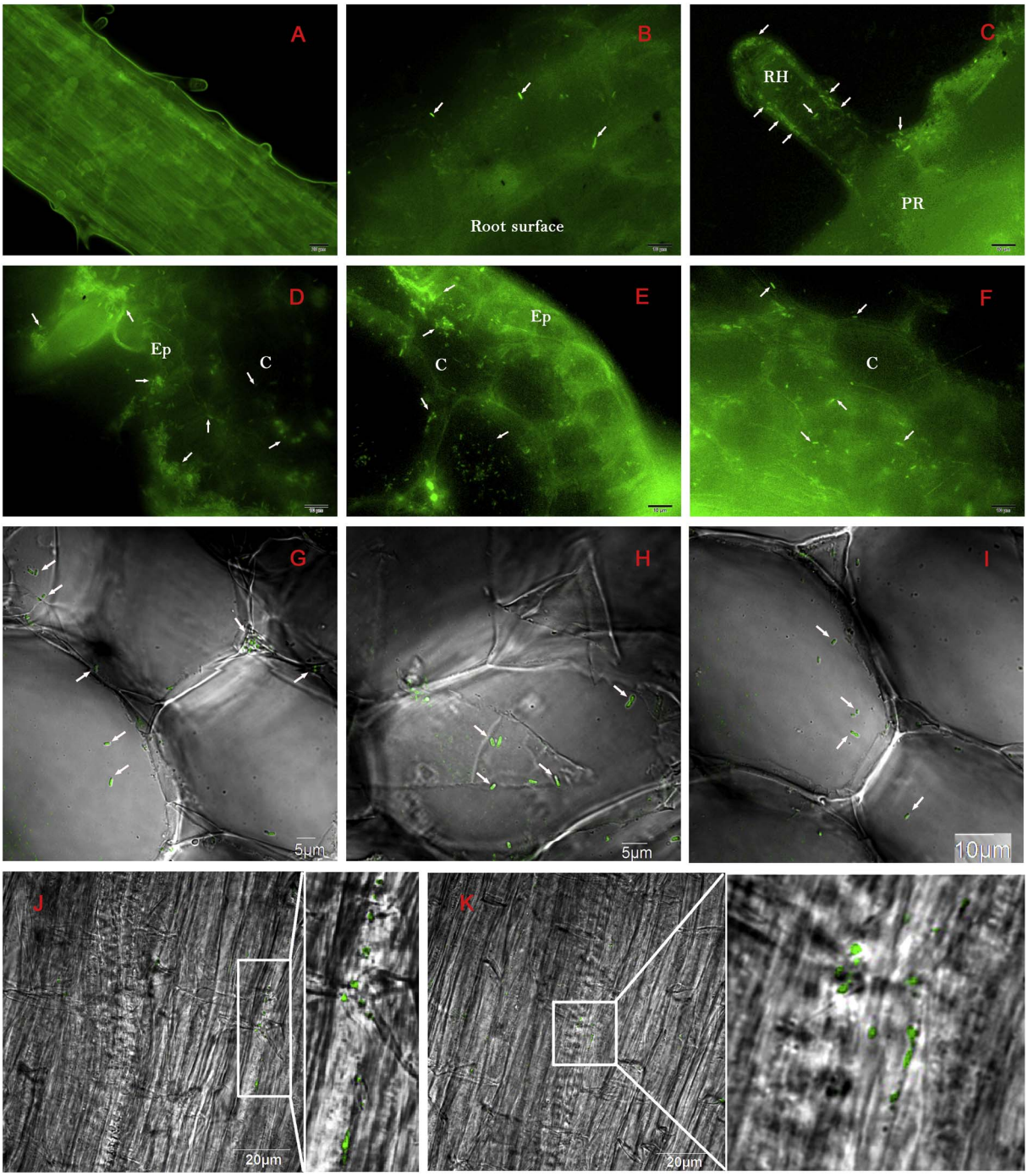

Fig. 2. Infection and colonization of *gfp*-tagged *P. agglomerans* SWg2 in mulberry seedlings. (A) Control of 0.85% saline water inoculation. (B, C) 2 DPI, Root hair and its emerged junction and root surfaces. (D) 3 DPI, Root hair emerged junction in primary root surface, cracks in rhizodermis and cellular space within epidermis. (E) 4 DPI, Exodermis, cortex and their cellular space in them. (F, G) 5 DPI, Cortex and inner cortex of primary root. (H, I) 7 DPI, Cortex, inner cortex and their cellular space of primary root. (J) 9 DPI, Xylem vessels in primary root. (K) 9 DPI, Pith in the central cylinder. Bacterial cells are indicated by *white arrows* (B–I) or *enlarged* (J, K). *Note:* PR: Primary root. RH: Root hair. Ep: Epidermis. C: Cortex.

4. Discussion

Mulberry bacterial blight also known as mulberry rotten head disease, caused by *Pseudomonas syringae* pv. *mori*, is a serious common disease worldwide on *Morus alba* L. (Young et al., 1978, 2001; Kuai, 2012). Increasing concern about the negative impaction of chemicals to the environment has facilitated an interest in the development of novel biocontrol methods (Getha et al., 2005). The present study investigated the control efficacy of the mulberry endophytic bacterium SWg2 against mulberry bacterial blight by pot experiments under greenhouse conditions. This *P. agglomerans* SWg2 strain had an effective role on suppression of bacterial blight caused by *P. syringae* pv. *mori*, since application of the cell-free supernatant or cell suspension of SWg2 strain both had relatively good control on the mulberry blight's development, which was similar or even better than the streptomycin (100 mg L^{-1}). Meanwhile, the safety evaluation experiment results indicated the *P. agglomerans* SWg2 had no side effects to the silkworm or its reproduction. On the contrary it showed the capability of protection the silkworm from the infection of pathogens. The application of SWg2 on plantlets beforehand or co-inoculation with the pathogen exhibited a more effective bio-control than the post treatment, suggesting that the SWg2 might represented an excellent competitor for

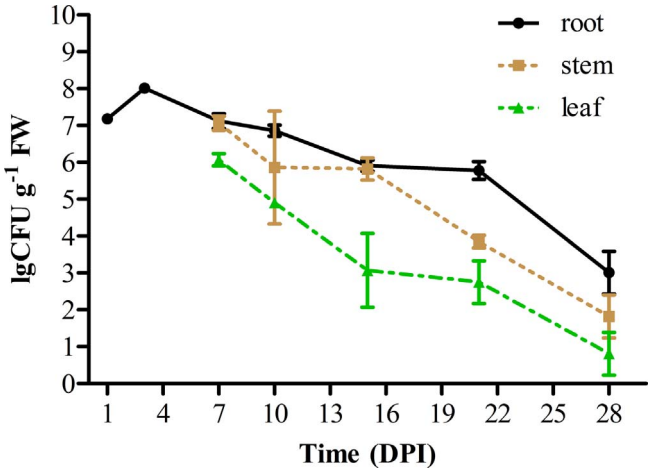

Fig. 3. Population dynamics of *gfp*-tagged *P. agglomerans* SWg2 in mulberry seedling.

Fig. 4. Evaluation the safety of *P. agglomerans* SWg2 to the silkworm of the 4th instar. (A) The pupae of silkworm which were fed the mulberry leaves with *P. agglomerans* SWg2 six times at the first and second day of the 4th instar stage. (B) The pupae of the synchronous feeding control group silkworm which did not be treated by the *P. agglomerans* SWg2. *Note:* Total 33 experimental silkworms in each group, and parts of them died at the 5th instar stage.

nutrients and niches compared with this pathogenic microorganism, since the utilization of carbon sources between *Pantoea agglomerans* and *P. syringae* are very much alike (Sammer et al., 2012). While it was reported that induced systemic resistance (ISR) might be one of the most important mechanisms of disease suppression triggered by inoculation with biocontrol bacteria (De Vleesschauwer et al., 2008; Kloeppe et al., 1999; Perazzolli et al., 2008; Siddiqui and Shaukat, 2002; Verhagen et al., 2004; Yedidia et al., 2003). We therefore suggest that induction of resistance in mulberry seedling might be one of the mechanisms underlying biocontrol by SWg2 strain as well, given that applied SWg2 prior to the inoculation of *P. syringae* pv. *mori*, the SWg2's populations were over the threshold necessary to trigger ISR (Siddiqui and Shaukat, 2002; Yedidia et al., 2003). Furthermore, Giddens et al. (2003) reported that the antibiotic AGA (alanylgriseoluteic acid) produced by *P. agglomerans* EH1087 (Giddens and Bean, 2007) significantly contributed to the competition with *Erwinia amylovora*, the pathogen caused fire blight in apple and pear. Likewise, antibiotic activity was observed by extracting and testing the active component of SWg2 against pathogenic organism (Xie unpublished). Thus, we speculate that the disease suppression of SWg2 might also be due to the production of an antibiotic resulting in antagonistic competition.

In this study, we also demonstrated that the *P. agglomerans* SWg2 could significantly promote the growth of mulberry seedlings, which the plumule length and radicle length of plants increased by large extent compared to the control. It has been reported that some plant beneficial microorganisms that produce IAA could promote root elongation resulting in shoot growth (Cattelan et al., 1999; Tanimoto, 2005). Sergeeva et al. (2007) confirmed that the inoculation of *Pantoea agglomerans* could distinctly stimulate the growth of pea, hyacinth bean and castor bean, probably due to the production of IAA enhancing the elongation and differentiation of roots. Our results demonstrated that SWg2 could synthesize IAA *in vitro* is consistent with the reports by Quecine et al. (2012) which indicated that endophytic *Pantoea agglomerans* 33.1 strain showed the capacity to produce IAA. Pandya and Saraf (2010) showed that synthesis of IAA in culture broth was largely dependent on the presence of tryptophan, and the density of IAA produced was also correlated with the amount of this amino acid. And the production of IAA by SWg2 was significantly enhanced in the culture medium containing L-tryptophan ($0.2\ g\ L^{-1}$), which corroborated the statements above.

Phosphorus is one of most limiting mineral nutrients influencing seedling development while is commonly in the insoluble existence such as calcium phosphates in soil, thus usually unavailable to plants (Rodríguez et al., 2006). Some plant growth-promoting microorganisms can dissolve mineral P and facilitate P uptake by plants to promote plant growth (Zaidi et al., 2006). Our results showed the SWg2 could dissolve tricalcium phosphate significantly, consequently increasing the amount of soluble phosphate in culture. Simultaneously, it was found that the pH of culture broth declined gradually during cultivation period, indicating that some organic acids might be secreted into culture by SWg2. The release of some organic acids by microorganisms might be the main mechanism of phosphate solubilization (Cunningham and Kuiack, 1992). However, unlike the endophytic *Pantoea* sp. 9 C (Loiret et al., 2004), *P. agglomerans* 33.1 (Quecine et al., 2012) strains of sugarcane and the endophyte *P. agglomerans* YS19 of rice (Feng et al., 2006), this *P. agglomerans* SWg2 strain from mulberry was negative for nitrogen fixation. This is probably due to strain differences and it is not and an unexpected result. Overall, the results above suggested that the growth-promoting traits of SWg2 is most likely due to the production of IAA as well as the ability of the strain to solubilize phosphate.

Many authors (Feng et al., 2006; Quecine et al., 2012) reported that the plant growth-promoting effect of *P. agglomerans* was correlated with its colonization on/in host plants. Rhizosphere colonization is believed as the critical progress for interaction between beneficial microbes and plants, contributing to plant growth promotion and suppression of some pathogens (Bais et al., 2006; Compant et al., 2010, 2005; Zhao et al., 2011). In this study, we explored the colonization of *P. agglomerans* SWg2 in mulberry plants under the combination of GFP-mark technology with enumeration after re-isolation. The GFP-tagged SWg2 cells were found attached to the emergence of root hair, root hair surface, and cracks on rhizodermis, in the form of aggregation, firstly, indicating that such bacteria might permeate through these import natural sites into plant. Once the bacteria entered the mulberry root they colonized the intercellular spaces of cortex and other tissues. Ultimately the bacterial cells successfully invaded the vascular bundles and spread to stem and leaf tissues. The population of SWg2-*gfp* colonized in roots

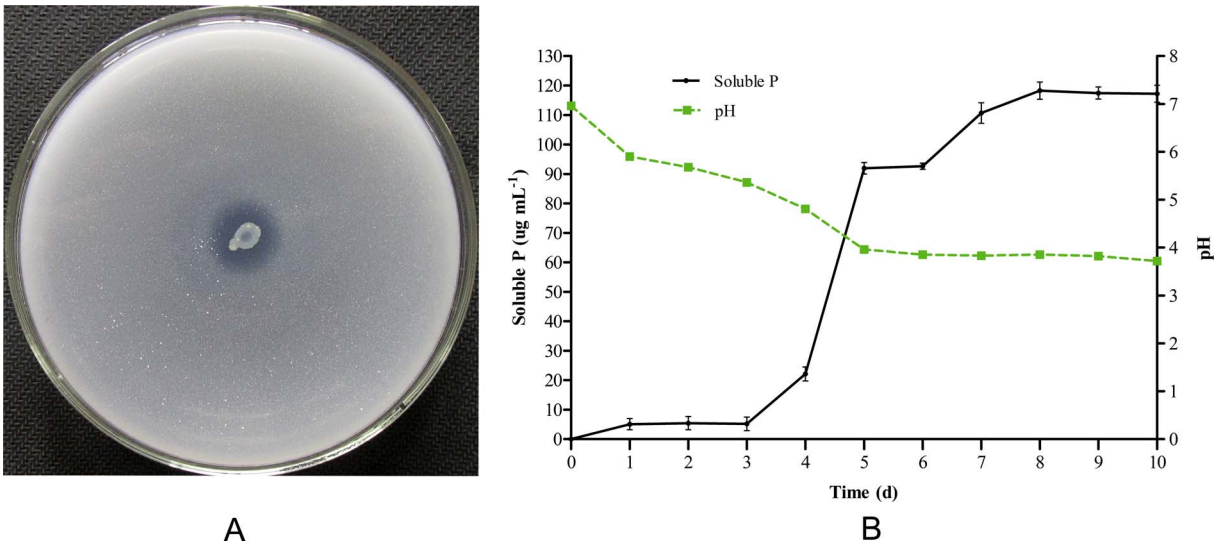

Fig. 5. Solubilization of insoluble inorganic phosphate $Ca_3(PO_4)_2$ by SWg2 strain. (A) Solubilization halo of $Ca_3(PO_4)_2$ generated by SWg2 strain. (B) Changes in phosphate released and pH over time during solubilization of $Ca_3(PO_4)_2$ by SWg2.

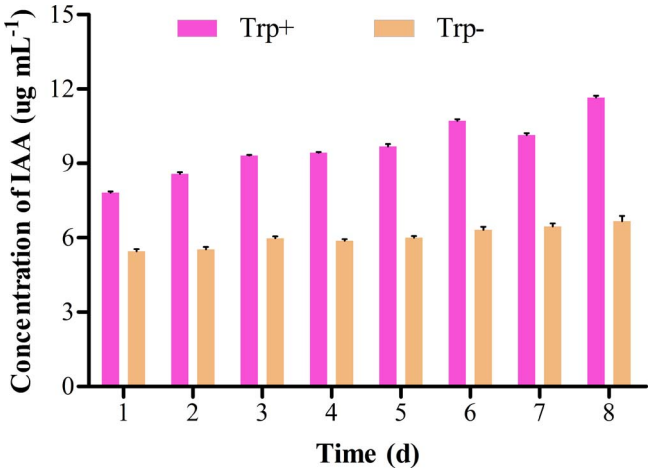

Fig. 6. Production of indole acetic acid (IAA) by *P. agglomerans* SWg2.

was the highest, more than that in stems, and the amount of bacteria within leaves was relatively the least. To our knowledge, this is the first report of colonization pattern for endophytic *P. agglomerans* SWg2 in mulberry, which is partly similar with other observations for some biocontrol strains (An et al., 2000; Ji et al., 2010, 2008; Liu et al., 2006, 2003; Meng et al., 2014). Therefore, our data confirmed that the SWg2 strain is an endophyte that can colonize the roots, stems, and leaves of mulberry plantlets using root immersion.

5. Conclusion

The present research results demonstrated that *P. agglomerans* SWg2 could successfully colonize different tissues of the mulberry plant. The data showed the promising beneficial role of *P. agglomerans* SWg2 on mulberry plantlets and it was able to effectively suppress the mulberry bacterial blight, and distinctively promote the growth of seedlings as well. Thus, *P. agglomerans* SWg2 was found to be a potentially biological control agent for mulberry bacterial blight, and also may act as a growth promoter.

Acknowledgments

The authors acknowledge the financial support of National Natural Science Foundation of China (31601678) to Jie Xie, Natural Science Foundation of Chongqing (cstc2015jcyjys80001) to Zhonghuai Xiang and Jie Xie, Fundamental Research Funds for the Central Universities (XDJK2017C039) to Jie Xie, and Postgraduate Research Innovation Project of Chongqing to Ping Shu (CYS14042).

References

An, Q., Yang, X., Dong, Y., Feng, L., Kuang, B., Li, J., 2000. Using confocal laser scanning microscope to visualize the infection of rice roots by GFP-labelled *Klebsiella oxytoca* SA2, an endophytic diazotroph. Acta Bot. Sin. 43, 558–564.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annu. Rev. Plant Biol. 57, 233–266.

Calderón, C.E., de Vicente, A., Cazorla, F.M., 2014. Role of 2-hexyl, 5-propyl resorcinol production by *Pseudomonas chlororaphis* PCL1606 in the multitrophic interactions in the avocado rhizosphere during the biocontrol process. FEMS Microbiol. Ecol. 89, 20–31.

Cattelan, A.J., Hartel, P.G., Fuhrmann, J.J., 1999. Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci. Soc. Am. J. 63, 1670–1680.

Chen, Y., Gao, X., Chen, Y., Qin, H., Huang, L., Han, Q., 2014. Inhibitory efficacy of endophytic *Bacillus subtilis* EDR4 against *Sclerotinia sclerotiorum* on rapeseed. Biol. Control 78, 67–76.

Compant, S., Clément, C., Sessitsch, A., 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol. Biochem. 42, 669–678.

Compant, S., Duffy, B., Nowak, J., Clément, C., Barka, E.A., 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. 71, 4951–4959.

Cunningham, J.E., Kuiack, C., 1992. Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium bilaii*. Appl. Environ. Microbiol. 58, 1451–1458.

De Vleesschauwer, D., Djavaheri, M., Bakker, P.A., Höfte, M., 2008. *Pseudomonas fluorescens* WCS374r-induced systemic resistance in rice against Magnaporthe oryzae is based on pseudobactin-mediated priming for a salicylic acid-repressible multifaceted defense response. Plant Physiol. 148, 1996–2012.

Erdogan, O., Benlioglu, K., 2010. Biological control of Verticillium wilt on cotton by the use of fluorescent *Pseudomonas* spp. under field conditions. Biol. Control 53, 39–45.

Feng, Y., Shen, D., Song, W., 2006. Rice endophyte *Pantoea agglomerans* YS19 promotes host plant growth and affects allocations of host photosynthates. J. Appl. Microbiol. 100, 938–945.

Gavini, F., Mergaert, J., Beji, A., Mielcarek, C., Izard, D., Kersters, K., De Ley, J., 1989. Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea* gen. nov. as *Pantoea agglomerans* comb. nov. and description of *Pantoea dispersa* sp. nov. Int. J. Syst. Bacteriol. 39, 337–345.

Getha, K., Vikineswary, S., Wong, W., Seki, T., Ward, A., Goodfellow, M., 2005. Evaluation of *Streptomyces* sp. strain g10 for suppression of Fusarium wilt and rhizosphere colonization in pot-grown banana plantlets. J. Ind. Microbiol. Biotechnol. 32, 24–32.

Giddens, S.R., Bean, D.C., 2007. Investigations into the in vitro antimicrobial activity and mode of action of the phenazine antibiotic D-alanylgriseoluteic acid. Int. J. Antimicrob. Agents 29, 93–97.

Giddens, S.R., Houliston, G.J., Mahanty, H.K., 2003. The influence of antibiotic production and pre-emptive colonization on the population dynamics of *Pantoea agglomerans* (*Erwinia herbicola*) Eh1087 and *Erwinia amylovora* in planta. Environ. Microbiol. 5,

1016–1021.
Glickmann, E., Dessaux, Y., 1995. A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl. Environ. Microbiol. 61, 793–796.
Graham, D.C., Hodgkiss, W., 1967. Identity of gram negative, yellow pigmented, fermentative bacteria isolated from plants and animals. J. Appl. Bacteriol. 30, 175–189.
Ji, X., Lu, G., Gai, Y., Gao, H., Lu, B., Kong, L., Mu, Z., 2010. Colonization of *Morus alba* L. by the plant-growth-promoting and antagonistic bacterium *Burkholderia cepacia* strain Lu10-1. BMC Microbiol. 10, 243.
Ji, X., Lu, G., Gai, Y., Zheng, C., Mu, Z., 2008. Biological control against bacterial wilt and colonization of mulberry by an endophytic *Bacillus subtilis* strain. FEMS Microbiol. Ecol. 65, 565–573.
Kloeppe, J., Rodriguez-Uabana, R., Zehnder, A., Murphy, J., Sikora, E., Fernandez, C., 1999. Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases. Aust. Plant Pathol. 28, 21–26.
Kuai, Y., 2012. Research progress in mulberry pathogenic prokaryotes and their diseases (I). Sci. Seric. 38, 152–164.
Kumar, V., Gupta, V., 2004. Scanning electron microscopy on the perithecial development of *Phyllactinia corylea* on mulberry – II sexual stage. J. Phytopathol. 152, 169–173.
Lam, B., Strobel, G.A., Harrison, L., Lam, S., 1987. Transposon mutagenesis and tagging of fluorescent *Pseudomonas*: antimycotic production is necessary for control of Dutch elm disease. Proc. Natl. Acad. Sci. U.S.A. 84, 6447–6451.
Lanna Filho, R., De Souza, R.M., Ferreira, A., Quecine, M.C., Alves, E., De Azevedo, J.L., 2013. Biocontrol activity of *Bacillus* against a GFP-marked *Pseudomonas syringae* pv. tomato on tomato phylloplane. Aust. Plant Pathol. 42, 643–651.
Liu, X., Zhao, H., Chen, S., 2006. Colonization of maize and rice plants by strain *Bacillus megaterium* C4. Curr. Microbiol. 52, 186–190.
Liu, Y., Chen, S., Li, J., 2003. Colonization pattern of *Azospirillum brasilense* Yu62 on maize roots. Acta Bot. Sin. 45, 748–752.
Loiret, F., Ortega, E., Kleiner, D., Ortega-Rodés, P., Rodés, R., Dong, Z., 2004. A putative new endophytic nitrogen-fixing bacterium *Pantoea* sp. from sugarcane. J. Appl. Microbiol. 97, 504–511.
Luo, J., Ran, W., Hu, J., Yang, X., Xu, Y., Shen, Q., 2010. Application of bio-organic fertilizer significantly affected fungal diversity of soils. Soil Sci. Soc. Am. J. 74, 2039–2048.
Meng, X., Yan, D., Long, X., Wang, C., Liu, Z., Rengel, Z., 2014. Colonization by endophytic *Ochrobactrum anthropi* Mn1 promotes growth of Jerusalem artichoke. Microb. Biotechnol. 7, 601–610.
Mizumoto, S., Hirai, M., Shoda, M., 2007. Enhanced iturin A production by *Bacillus subtilis* and its effect on suppression of the plant pathogen *Rhizoctonia solani*. Appl. Microbiol. Biotechnol. 75, 1267–1274.
Pandya, U., Saraf, M., 2010. Application of fungi as a biocontrol agent and their biofertilizer potential in agriculture. J. Adv. Dev. Res. 1, 90–99.
Patten, C.L., Glick, B.R., 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol. 68, 3795–3801.
Perazzolli, M., Dagostin, S., Ferrari, A., Elad, Y., Pertot, I., 2008. Induction of systemic resistance against *Plasmopara viticola* in grapevine by *Trichoderma harzianum* T39 and benzothiadiazole. Biol. Control 47, 228–234.
Pidot, S.J., Coyne, S., Kloss, F., Hertweck, C., 2014. Antibiotics from neglected bacterial sources. Int. J. Med. Microbiol. 304, 14–22.
Pratibha, V., Praveen, R., Anjali, C., Arvind, G., 2007. Phosphate solubilization potential and stress tolerance of *Eupenicillium parvum* from tea soil. Mycol. Res. 111, 931–938.
Quecine, M.C., Araujo, W.L., Rossetto, P.B., Ferreira, A., Tsui, S., Lacava, P.T., Mondin, M., Azevedo, J.L., Pizzirani-Kleiner, A.A., 2012. Sugarcane growth promotion by the endophytic bacterium *Pantoea agglomerans* 33.1. Appl. Environ. Microbiol. 78, 7511–7518.
Rodríguez, H., Fraga, R., Gonzalez, T., Bashan, Y., 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287, 15–21.
Rösch, C., Mergel, A., Bothe, H., 2002. Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. Appl. Environ. Microbiol. 68, 3818–3829.
Ryan, R.P., Germaine, K., Franks, A., Ryan, D.J., Dowling, D.N., 2008. Bacterial endophytes: recent developments and applications. FEMS Microbiol. Lett. 278, 1–9.
Sammer, U.F., Reiher, K., Spiteller, D., Wensing, A., Völksch, B., 2012. Assessment of the relevance of the antibiotic 2-amino-3-(oxirane-2, 3-dicarboxamido)-propanoyl-valine from *Pantoea agglomerans* biological control strains against bacterial plant pathogens. Microbiol. Open 1, 438–449.
Sammer, U.F., Völksch, B., Möllmann, U., Schmidtke, M., Spiteller, P., Spiteller, M., Spiteller, D., 2009. 2-Amino-3-(oxirane-2,3-dicarboxamido)-propanoyl-valine, an effective peptide antibiotic from the epiphyte *Pantoea agglomerans* 48b/90. Appl. Environ. Microbiol. 75, 7710–7717.
Sergeeva, E., Hirkala, D.L.M., Nelson, L.M., 2007. Production of indole-3-acetic acid, aromatic amino acid aminotransferase activities and plant growth promotion by *Pantoea agglomerans* rhizosphere isolates. Plant Soil 297, 1–13.
Siddiqui, I.A., Shaukat, S.S., 2002. Rhizobacteria-mediated induction of systemic resistance (ISR) in tomato against *Meloidogyne javanica*. J. Phytopathol. 150, 469–473.
Tanimoto, E., 2005. Regulation of root growth by plant hormones-roles for auxin and gibberellin. Crit. Rev. Plant Sci. 24, 249–265.
Tian, L., Li, X., 1996. Evaluation of resistant of mulberry germplasm resources to *Pseudomonas syingae* pv. *mori* van hall. Acta Sericol. Sin. 22, 205–207.
Verhagen, B.W., Glazebrook, J., Zhu, T., Chang, H.S., Van Loon, L.C., Pieterse, C.M., 2004. The transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis*. Mol. Plant Microbe Interact. 17, 895–908.
Yedidia, I., Shoresh, M., Kerem, Z., Benhamou, N., Kapulnik, Y., Chet, I., 2003. Concomitant induction of systemic resistance to *Pseudomonas syringae* pv. *lachrymans* in cucumber by *Trichoderma asperellum* (T-203) and accumulation of phytoalexins. Appl. Eviron. Microbiol. 69, 7343–7353.
Young, J.M., Bull, C.T., De Boer, S.H., Firrao, G., Gardan, L., Saddler, G.E., Stead, D.E., Takikawa, Y., 2001. Classification, nomenclature, and plant pathogenic bacteria – a clarification. Phytopathology 91, 617–620.
Young, J.M., Dye, D.W., Bradbury, J.F., Panagopoulos, C.G., Robbs, C.F., 1978. A proposed nomenclature and classification for plant pathogenic bacteria. N. Z. J. Agric. Res. 21, 153–177.
Zaidi, S., Usmani, S., Singh, B.R., Musarrat, J., 2006. Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. Chemosphere 64, 991–997.
Zhang, F., Gao, Y., Ren, H., Qiu, L., Zuo, W., Zhou, Z., Xie, J., 2013. Screening, identification and optimization of fermentation conditions of an antagonistic endophyte to mulberry bacterial blight. Acta Microbiol. Sin. 53, 1285–1294.
Zhao, Q., Shen, Q., Ran, W., Xiao, T., Xu, D., Xu, Y., 2011. Inoculation of soil by *Bacillus subtilis* Y-IVI improves plant growth and colonization of the rhizosphere and interior tissues of muskmelon (*Cucumis melo* L.). Biol. Fertil. Soils 47, 507–514.