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Application of antagonistic rhizobacteria for control of Fusarium seedling blight and basal rot of lily

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Abstract Three antagonistic bacteria, *Streptomyces misionensis* strain PMS101, *Bacillus thermoglucosidasius* strain PMB207, and *S. sioyaensis* strain PMS502, were tested for sensitivity to the foliar fungicide Sporgon (a.i. 50% prochloraz-Mn complex) and for efficacy in controlling Fusarium diseases of lily. Results showed that the growth of all three antagonistic strains of bacteria was completely suppressed by Sporgon at a concentration of 500 μg/mL, but *B. thermoglucosidasius* strain PMB207 and *S. misionensis* strain PMS101 were unaffected at concentrations of 100 μg/mL or lower. A large-scale trial in an automated and environment-controlled commercial greenhouse showed that treatment of scale bulblets of lily with Sporgon (100 μg/mL) and *B. thermoglucosidasius* strain PMB207 (1–1.2×10^7 cfu/mL) or 100 μg/mL Sporgon and *S. misionensis* strain PMS101 (1–1.4×10^7 cfu/mL) resulted in a significant reduction ($P<0.05$) in the incidence of seedling blight caused by *Fusarium proliferatum*. The difference between these two treatments was not significant ($P>0.05$). Results of the greenhouse and field experiments showed that treatment of scale bulblets or one-year-old bulbs of lily with *B. thermoglucosidasius* strain PMB207 (1–1.2×10^8 cfu/mL) or *S. misionensis* strain PMS101 (1–1.4×10^8 cfu/mL) without Sporgon was also effective in the control of basal rot caused by *F. oxysporum* f. sp. *lilii*. These studies reveal that *B. thermoglucosidasius* strain PMB207 and *S. misionensis* strain PMS101 are biocontrol agents which have potential for use in the commercial production of lily bulbs, as they can be used alone or in combination with the fungicide Sporgon at low concentration (<100 μg/mL).

Keywords *Fusarium proliferatum* · Lily seedling blight · *F. oxysporum* f. sp. *lilii* · Lily basal rot · Sporgon · Antagonistic bacteria · *Streptomyces misionensis* · *Bacillus thermoglucosidasius*

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Introduction

Planting lily (*Lilium* L.) in soilless culture is considered a suitable method to reduce risks of diseases caused by soilborne pathogens (Tribulato and Noto 2001; Tribulato et al. 2003). In Taiwan, basal rot caused by *Fusarium oxysporum* f. sp. *lilii* (Fol.) and seedling blight caused by *F. proliferatum* (Matsushima) Nirenberg are the two major diseases of lily affecting production of this crop due to continuous monoculture practice in the field or use of different non-sterile soilless culture media in commercial greenhouses (Lee and Leu 1998). Symptoms of basal rot

of lily are root rot and basal plate rot of the bulb, leaf chlorosis, defoliation, stunting and wilt (Löffler et al. 1995; Baayen and Rijkenberg 1999). Symptoms of seedling blight of lily are damping-off and leaf yellowing and necrosis of bulbs and roots (Wu et al. 1998; Prados-Ligero et al. 2008). Methods for control of these diseases include the use of fungicides such as Sporgon (a.i.50% prochloraz-Mn complex) (Riedel-deHaën, Co., AG, Seelze, Germany) for the treatment of lily bulbs. Other control methods for this disease include pasteurization of soil with aerated steam (Lee and Leu 1998), modification of cultural practices (Lawson and Hsu 1996), use of antifungal substances extracted from bulbs and/or roots of the genus *Lilium* (Lim et al. 2005) and use of disease resistant cultivars (Straathof et al. 1993, 1997; Straathof and Löffler 1994; Lim et al. 2003). Although disease resistance is the most effective method for the management of Fusarium diseases of lily, this method is complicated due to the presence of races of these pathogens in different countries.

Several antagonistic bacteria and fungi have been used for control of Fusarium wilt of various crops, including cyclamen (*Cyclamen persicum* Miller) (Minuto et al. 1995), tomato (*Solanum lycopersicum* L.) (Larkin and Fravel 1999), garden asparagus (*Asparagus officinalis* L.) (He et al. 2002), and pigeon pea (*Cajanus cajan* (L.) Millsp.) (Bapat and Shah 2000). However, no information is available on control of Fusarium diseases of lily by antagonistic bacterial agents alone or combined use of biocontrol agents and fungicide. The objective of this study was to determine the effectiveness of antagonistic bacteria alone or in combination with the fungicide Sporgon (a.i. 50% prochloraz-Mn complex) in controlling Fusarium diseases of lily in a commercial greenhouse using soilless culture and in fields naturally-infested with the pathogen.

Methods

Fungal pathogens, plant material and commercial culture medium

Fusarium oxysporum f. sp. *lilii*, strain G16, and *Fusarium proliferatum*, strain FP08, were obtained from diseased plants of Asiatic lily, hybrid cultivar Acapulco. Strain G16 was from Hsingshe county and strain FP08 was from Tali county of Taichung, Taiwan. Both strains were highly virulent to lily (Wu et al. 1998; Huang et al. 2005). Stock cultures were stored in sterilized beads at −80°C using the method of Belkacemi et al. (1997). Each strain was grown on potato dextrose agar (PDA, Difco, USA) for 7 days and used as working cultures. Spore suspensions (8×10^8 cfu/mL) were prepared and mixed with soil or BVB No. 4 (Bas Van Burren, Visser, Netherland, 20% black peat, 10% sand, and 70% white German peat at a pH of 5.6–6.0 ($N:P_2O_5:K_2O$: 130:110:230 mg l^{-1})) at a ratio of 1:100 (v/w) using the method of Straathof et al. (1993). Four weeks later, the number of propagules in the infested soil or BVB No.4 was determined by a dilution plate method, using a semiselective medium (1.0 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4$, 0.01 g Fe-Na-EDTA, 2.0 gL-asparagine, 20.0 g galactose, 1.0 g pentachloronitrobenzene, 0.5 g oxgall, 1.0 g $Na_2B_4O_7.10H_2O$, 0.3 g streptomycin sulfate, 0.1 g benomyl, 15.0 g agar and 1.0 L distilled water, pH 4.0) developed by Huang et al. (2005). Healthy bulbs of Asiatic lily, hybrid cultivar Casa Blanca, used in all the experiments were supplied by the Seed Propagation and Improvement Station, Hsingshe, Taichung, Taiwan and the Sugar Research Institute, Tainan, Taiwan.

Maintenace of antagonistic rhizobacteria

Three strains of antagonistic rhizobacteria, *Bacillus thermoglucosidasius* strain PMB207, *Streptomyces misionensis* strain PMS101 and *S. sioyaensis* strain PMS502, were isolated from the rhizosphere of healthy lily bulbs and used in this study. *Bacillus thermoglucosidasius* and *S. misionensis* were collected from Tali county and *S. sioyaensis* was from Wufeng county, Taichung, Taiwan. The media used for culturing these bacterial strains were PDA for *B. thermoglucosidasius* and ISP4 (International Streptomyces Project No.4, Difco, USA) for *S. misionensis* and *S. sioyaensis*. Stock cultures were lyophilized and stored at −20°C.

Sensitivity of antagonistic rhizobacteria to Sporgon

For testing sensitivity of rhizobacteria to the fungicide Sporgon (a.i. 50% prochloraz-Mn complex), autoclaved PDA was cooled to about 60°C before adding Sporgon at concentrations of 0, 10, 50, 100 and 500 μg/mL, and poured immediately in Petri dishes, 20 mL/dish. After cooling for 2–4 h, the dishes were inoculated individually with 2 μl conidial suspensions of *S. misionensis* strain PMS101 ($1–1.2\times10^5$ cfu/mL) or *S. sioyaensis* strain PMS502 ($1.8–2.1\times10^5$ cfu/mL) from 7-day-old cultures or 2 μl bacterial suspensions of *B. thermoglucosidasius* strain PMB207 ($1.8–2.1\times10^5$ cfu/mL) from 2-day-old cultures. The concentration of all bacterial suspensions was determined by measurement of optical density.. After incubation at 28°C for 5 days, the number of colonies developed in each dish was recorded. For each experiment, there were four replicates in each treatment. The experiment was conducted once.

Effect of antagonistic rhizobacteria on control of lily seedling blight caused by *Fusarium proliferatum* (Greenhouse experiments)

Scale bulblets of lily, cv. Casa Blanca, were surface-sterilized with 1% sodium hypochlorite for 2 min, rinsed twice with sterile distilled water and soaked for 2 min in 500 mL suspensions of *S. misionensis* strain PMS101 (1–1.4×10^7 cfu/mL), *S. sioyaensis* strain PMS502 (1–1.4×10^7 cfu/mL) and *B. thermoglucosidasius* strain PMB207 (1–1.2×10^7 cfu/mL), respectively. Scale bulblets treated with sterile distilled water were used as controls. Plastic containers (11×8.5 cm, diameter × height) were filled with BVB No.4 medium infested with *F. proliferatum* (1–1.8×10^4 cfu per gram of medium). For each replicate, twenty scale bulblets were buried in the BVB No.4 medium at a depth of 2 cm. Plants were held in the greenhouse at 24–28°C, and watered daily. There were four containers in each treatment. Fresh weight of plants and bulblets, and disease severity were recorded at 8 weeks after planting. A disease severity index (DI) for each replicate of each treatment was calculated using the formula: $DI = \Sigma(nR)/T$ (n = number of bulbs (or bulblets) affected by disease; R = disease rating 0–5, where 0 = healthy bulbs (or bulblets), 1 = 10%–30% of the bulbs (or bulblets) with decay, 2 = 31%–50% of the bulbs (or bulblets) with decay, 3 = 51%–70% of the bulbs (or bulblets) with decay, 4 =71%–90% of the bulbs (or bulblets) with decay, and 5 = bulbs (or bulblets) completely decayed; T = total number of bulbs (or bulblets)). The experiment was conducted twice.

Control of lily seedling blight by Sporgon and rhizobacteria (Greenhouse experiments)

Scale bulblets, cv. Casa Blanca, were surface-sterilized with 1% sodium hypochlorite for 2 min, rinsed twice with sterile distilled water and soaked in Sporgon (100 μg/mL) for 10 min at room temperature (26–30°C). After being air-dried, bulblets were dipped in a suspension of the Sporgon-tolerant antagonistic rhizobacteria, *S. misionensis* strain PMS101 (1–1.4×10^7 cfu/mL), *S. sioyaensis* strain PMS502 (1–1.4×10^7 cfu/mL) and *B. thermoglucosidasius* strain PMB207 (1–1.2×10^7 cfu/mL), respectively, for 2 min. Scale bulblets treated with Sporgon or sterile distilled water were used as controls. Plastic containers (30×15×12 cm, L × W × H) were filled with BVB No. 4 media infested by *F. proliferatum* (1–1.8×10^4 cfu per gram of medium). Scale bulblets were planted in pathogen-infested or untreated (control) media at a depth of 2 cm, with 20 bulblets per replicate. Plants were held in the greenhouse at 24–28°C and watered daily. There were four containers for each treatment. Disease severity was recorded 8 weeks after planting using the method described previously. The experiment was conducted twice.

Control of lily seedling blight by combined treatment of Sporgon and rhizobacteria (Large-scale greenhouse experiments in BVB No. 4)

The commercial growth medium BVB No.4 was adjusted to 50%–60% moisture content, artificially infested with *F. proliferatum* (1–1.8×10^4 cfu per gram of medium) in a medium mixer (Visser Co., Netherlands) for 15 min., and then used to fill containers (2.8×1.2×0.2 m, L × W × H) (Alcoa Co., Netherlands) using a medium filler (Visser Co., Netherlands). Lily scale bulblets, cv. Casa Blanca were treated with Sporgon (100 μg/mL), followed by treatment with either a suspension of *S. misionensis* strain PMS101 (1–1.4×10^7 cfu/mL) or *B. thermoglucosidasius* strain PMB207 (1–1.2×10^7 cfu/mL). The scale bulblets were then planted in the medium in containers by the same method described above. The containers were held in an automated environment-controlled greenhouse at 25–32°C, 60%–90% relative humidity, and 400–600 $W.m^{-2}$ light density with a wavelength range of 400–1,100 nm. Untreated scale bulblets were used as controls. There were four replicates (containers) in each treatment, with 1,000 bulblets in each container. The incidence of seedling blight of lily and fresh weight of seedlings were recorded two months after planting. The experiment was conducted twice.

Control of lily basal rot by antagonistic rhizobacteria (Greenhouse experiments in soil)

Scale bulblets of lily, cv. Casa Blanca, were surface-sterilized with 1% sodium hypochlorite for 2 min, rinsed twice with sterile distilled water and soaked for 30 min in a suspension of *S. misionensis* strain PMS101 (1–1.4×10^8 cfu/mL) and *B. thermoglucosidasius* strain PMB207 (1–1.2×10^8 cfu/mL), respectively. Sandy loam soils collected from Tali, Taichung, Taiwan, were artificially infested with *F. oxysporum* f. sp. *lilii* (1×10^3 cfu per gram of soil) and used to fill pots (8-cm diameter). Five scale bulblets were planted in each pot at a depth of 2.0 cm. Untreated scale bulblets planted in untreated soil were used as controls. Plants were held in the greenhouse at 24–28°C and watered daily. Disease severity was recorded at 8 and 20 weeks after planting using the method described above. The experiment was conducted twice. For each experiment, there were four pots in each treatment.

Control of lily basal rot by antagonistic rhizobacteria (Field experiments)

Two experiments were conducted from 16 Nov. 1998 to 16 May 1999 in the fields near Chihu, Chang-Hua, Taiwan.

Table 1 Effect of the fungicide Sporgon on growth of three strains of antagonistic rhizobacteria

Antagonistic rhizobacteria	Sporgon concentration (μg ml^{-1})				
	0	10	50	100	500
S. misionensis PMS101	57±3.2^{z} a^{y}	55±2.3 a	56±1.2 a	56±1.7 a	0 b
S. sioyaensis PMS502	51±1.9 a	52±2.5 a	52±1.6 a	39±2.9 b	0 c
B. thermoglucosidasius PMB207	65±1.5 a	64±3.7 a	65±3.1 a	63±2.9 a	0 b

^{z} Data presented are colony forming units (cfu ml^{-1}) ± standard error. Data were collected after incubation of the cultures on PDA at 28°C for 5 days

^{y} Means within each row followed by the same letter are not significantly different (P>0.05) according to Tukey's multiple range test

The fields were naturally infested with *F. oxysporum* f. sp. *lilii* (7.2×10^4 cfu/ml in Chihu and 9.2×10^4 cfu/ml in Chang-Hua) detected by a semi-selective medium developed by Huang et al. (2005). Scale bulblets and 1-year-old bulbs from scale bulblets of lily, cv. Casa Blanca, were surface-sterilized with 1% sodium hypochlorite for 2 min, rinsed twice with sterile distilled water and soaked for 30 min in a suspension of *S. misionensis* strain PMS101 (1–1.4×10^8 cfu/mL), and *B. thermoglucosidasius* strain PMB207 (1–1.2×10^8 cfu/mL), respectively. The scale bulblets and one-year-old bulbs treated with antagonistic rhizobacteria were planted into the soil at a depth of 15–20 cm (10 cm of spacing between bulblets) in the fields near Chihu, with 1,500 bulblets per plot. For each field, there were three treatments including two antagonistic microorganisms and one untreated control. Treatments were arranged in a completely randomized design with four replicates per treatment. Each replicate plot consisted of a single raised row (25×0.85×0.3 m, L × W × H) prepared according to the commercial practices of lily production. The row orientation was north to south. Emergence of scale bulblets and one-year-old bulbs for each replicate of each treatment was determined at 4 weeks after planting and data on incidence of basal rot, circumference of bulbs and recovery of bulbs of lily were recorded at 6 months after planting by counting the plants at the centre of each row for an area of 3×0.85 m (L × W). There were four replicates for each treatment.

Statistical analysis

Data on growth of antagonistic rhizobacteria, fresh weight of bulblets and lily plants, seedling emergence, recovery of bulbs, circumference of bulbs, and disease severity index, were collected from the experiments and were analyzed using analysis of variance (ANOVA). Data from repeats of the same experiment were pooled when the variances were homogenous. Statistical significance was tested with untransformed data of seedling emergence and recovery of bulbs or arcsine square root-transformed data of disease index. Tukey's multiple range test (TMRT) (SAS Institute

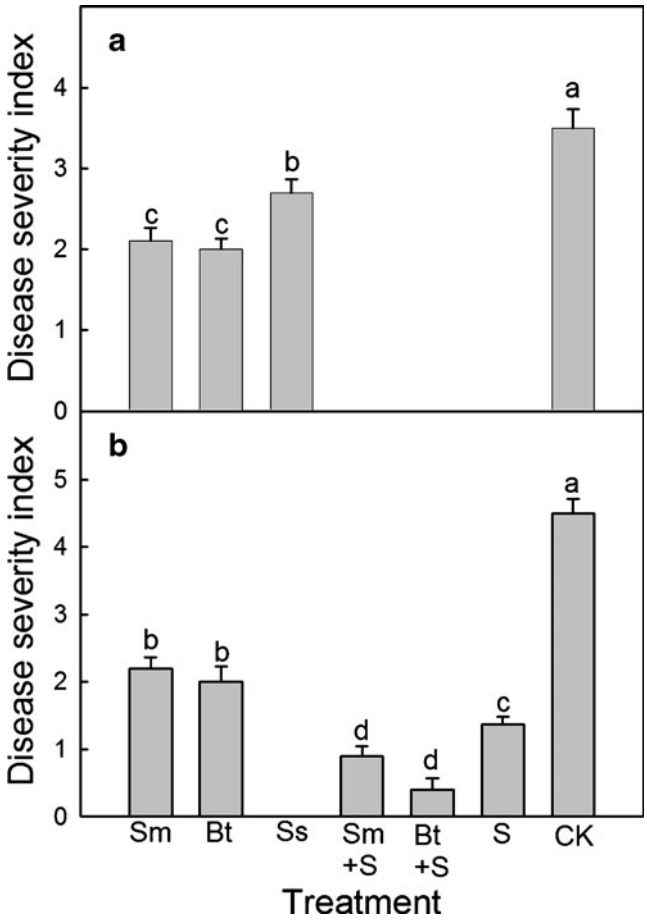

Fig. 1 Effect of treatment of scale bulblets with rhizobacteria alone (**a**) or in combination with the fungicide Sporgon (**b**) on the severity of seedling blight of lily, cv. Casa Blanca (Greenhouse experiments). Treatments are: Sm = *S. misionensis* strain PMS101; Bt = *B. thermoglucosidasius* strain PMB207; Ss = *S. sioyaensis* strain PMS502; Sm + S = Sm + Sporgon; Bt + S = Bt + Sporgon; S = Sporgon; CK = Control. Disease severity index was recorded at 8 weeks after planting. The disease severity index data (mean of the two experiments) were arcsine transformed prior to analysis. For each figure, means followed by the same letter are not significantly different (P> 0.05) according to Tukey's multiple range test. Error bars are standard deviations. Disease severity index was measured according to a scale 0–5, where 0 = healthy bulbs (or bulblets); 1 = 10%–30% of the bulbs (or bulblets) with decay; 2 = 31%–50% of the bulbs (or bulblets) with decay; 3 = 51%–70% of the bulbs (or bulblets) with decay; 4 = 71%–90% of the bulbs (or bulblets) with decay; 5 = completely decayed

Inc.) was used to compare means of the treatments in each experiment.

Results

Sensitivity of antagonistic rhizobacteria to Sporgon

Results of laboratory tests showed that growth of the rhizobacteria, *S. misionensis* PMS101 and *B. thermoglucosidasius* PMB207 was unaffected ($P>0.05$) on PDA amended with the fungicide Sporgon at the concentrations of 10–100 μg/mL (Table 1) but growth of *S. sioyaensis* PMS502 was significantly reduced ($P<0.05$) by the treatment of Sporgon at 100 μg/mL. For the treatment of Sporgon at 500 μg/mL, growth was completely suppressed in all the three strains of rhizobacteria tested.

Effect of antagonistic rhizobacteria alone or combined with Sporgon on control of seedling blight of lily in soilless culture media

Results of the experiments showed that *S. misionensis* strain PMS101 and *B. thermoglucosidasius* strain PMB207 were the most effective agents in reducing severity of seedling blight of lily caused by *F. proliferatum* (Fig. 1a). Although the disease reduction by *S. sioyaensis* strain PMS502 was also significant ($P<0.05$), it was less effective than *S. misionensis* strain PMS101 and *B. thermoglucosidasius* strain PMB207.

Treatment of scale bulblets with Sporgon (100 μg/mL) alone was significantly better than the treatment of *S. misionensis* strain PMS101 ($1–1.4\times10^7$ cfu/mL) or *B. thermoglucosidasius* strain PMB207 ($1–1.2\times10^7$ cfu/mL) in the control of seedling blight of lily (Fig. 1b). However, a combined treatment of scale bulblets with 100 μg/ml Sporgon and *B. thermoglucosidasius* strain PMB207 or 100 μg/mL Sporgon and *S. misionensis* strain PMS101 was more effective than the treatment of fungicide Sporgon alone or any of these two rhizobacterial strains alone. Neverthless, there was no significant difference between the treatment of Sporgon + *B. thermoglucosidasius* strain PMB207 and Sporgon + *S. misionensis* strain PMS101 (Fig. 1b).

Results of the large scale trial in soilless culture in a commercial greenhouse also showed that the combined treatments of Sporgon and rhizobacteria were effective in reducing severity of seedling blight of lily (Table 2). Both *B. thermoglucosidasius* strain PMB207 and *S. misionensis* strain PMS101 significantly reduced disease severity and increased seedling fresh weight, although *B. thermoglucosidasius* strain PMB207 had the greatest effects (Table 2).

Control of lily basal rot in greenhouse and field experiments by antagonistic rhizobacteria

Results of greenhouse experiments in soil artificially infested with *F. oxysporum* f. sp. *lilii* showed that treatment of scale bulblets with *B. thermoglucosidasius* strain PMB207 or *S. misionensis* strain PMS101 significantly reduced ($P<0.05$) basal rot of lily, based on the data collected at eight and 20 weeks after planting (Fig. 2). Results of experiments in field plots naturally infested with *F. oxysporum* f. sp. *lilii* confirmed the efficacy of control of basal rot of lily by *B. thermoglucosidasius* strain PMB207 and *S. misionensis* strain PMS101 (Table 3). *F. oxysporum* f.sp. *lilii* was reisolated from diseased lily bulbs in this experiment. Treatment with *B. thermoglucosidasius* strain PMB207 or *S. misionensis* strain PMS101 not only significantly increased ($P<0.05$) seedling emergence and scale bulblet recovery but also significantly reduced ($P<0.05$) disease severity in scale bulblets. However, these two treatments did not cause a significant increase in the circumference of lily bulbs. For the one-year-old bulbs, the treatment of *S. misionensis* strain PMS101 resulted in a significant reduction in disease severity, whereas treatment with *S. misionensis* strain PMS101 and *B. thermoglucosidasius* strain PMB207 resulted in a significant increase in the circumference of lily bulbs (Table 3).

Table 2 Effect of treatment of scale bulblets with Sporgon and antagonistic rhizobacteria on control of seedling blight of lily (Large-scale commercial greenhouse experiment)

Treatment	Disease severity index (0–5)[z]	Fresh weight (g/100 plants)[y]
S. misionensis PMS101+ Sporgon	1.9 b[x]	138 b
B. thermoglucosidasius PMB207 + Sporgon	0.9 c	155 a
Control	3.7 a	95 c

[z] Disease severity index was recorded 2 months after planting based on a disease rating scale of 0–5, while 0 = healthy and 5 = plant completely decayed. The disease severity index data (mean of the two experiments) were arcsine transformed prior to analysis

[y] Fresh weight of plants was recorded 2 months after planting

[x] Means within each column followed by the same letter are not significantly different ($P>0.05$) according to Tukey's multiple range test

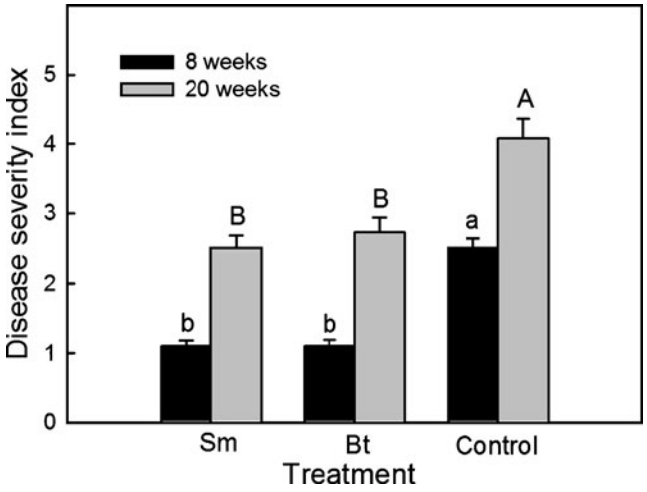

Fig. 2 Effect of treatment of scale bulblets with antagonistic rhizobacteria on severity of basal rot of lily caused by *Fusarium oxysporum* f. sp. *lilii* in the field soil. Sm = *S. misionensis* and Bt = *B. thermoglucosidasius*. Data (mean of the two experiments) were arcsine square root transformed prior to the analysis. Treatments followed by the same letter are not significantly different ($P>0.05$) according to Tukey's multiple range test. Error bars are standard deviations. Disease severity index was the basal rot of bulbs measured according to a scale 0–5, where 0 = healthy bulbs (or bulblets); 1 = 10%–30% of the bulbs (or bulblets) with decay; 2 = 31%–50% of the bulbs (or bulblets) with decay; 3 = 51%–70% of the bulbs (or bulblets) with decay; 4 = 71%–90% of the bulbs (or bulblets) with decayed; 5 = completely decayed

Discussion

Numerous reports indicate that some fungicides are harmful to beneficial microorganisms such as *Trichoderma* species (Khan and Shahzad 2007), *Azospirillum* sp. (Mubeen et al. 2006), *Bradyrhizobium japanicum* (Revellin et al. 1993) and *Pseudomonas fluorescens* (Malathi et al. 2002). Thus, compatibility between pesticides and antagonistic microorganisms may be an important factor affecting efficacy of control of soilborne diseases by the combined treatment of fungicides and microbial agents (O'Neill 1995). However, the present study reveals that the fungicide Sporgon at a concentration of 100 μg/mL or lower is not harmful to *S. misionensis* strain PMS101 and *B. thermoglucosidasius* strain PMB207 (Table 1), and that the combined treatment is effective in the control of Fusarium seedling blight of lily (Fig. 1). Since Sporgon is commonly used as a soil drench treatment at 400 μg/mL to control Fusarium seedling blight of lily in Taiwan, the effective control of this disease by a combined treatment of antagonistic bacteria and Sporgon (Fig. 1) represents a substantial reduction of use of this chemical pesticide. Therefore, this combined treatment method would be more practical as it is more environmentally friendly than the current treatment of Sporgon at 400 μg/mL alone. The finding in this study confirms previous reports that combined treatments of biocontrol agents and a reduced rate of fungicides are effective for control of soilborne pathogens in various crops (Zhou and Reeleder 1990; Chand-Goyal and Spotts 1996; Conway et al. 1997; Budge and Whipps 2001; Omar et al. 2006).

Pham et al. (2004) reported that pesticides may cause specific stresses on bacterial cells such as damage to proteins, oxidative DNA, or cell membranes. This phenomenon might have occurred in relation to *S. misionensis* strain PMS101 and *B. thermoglucosidasius* strain PMB207 used in this study as Sporgon at 500 μg/mL completely suppressed the growth of these two rhizobacterial strains.

Table 3 Control of Fusarium basal rot of lily by treatment of scale bulblets and one-year-old bulbs with antagonistic rhizobacteria (Field experiments)

Treatment	Emergence (%)[z]		Recovery (%)[y]		Circumference (cm)[y]		Disease severity index[x]	
	Scale bulblet	1-year-old bulb	Scale bulblet	1-year-old bulb	Scale bulblet	1-year-old bulb	Scale bulblet	1-year-old bulb
Streptomyces misionensis PMS101	84.0 a[w]	84.0 a	67.0 a	77.0 a	6.9 a	12.2 a	1.9 b	0.9 b
Bacillus thermoglucosidasius PMB207	85.0 a	90.0 a	70.0 a	75.0 a	6.7 a	11.9 a	1.4 b	1.2 a
Control [v]	69.0 b	81.0 a	49.0 b	73.0 a	6.4 a	11.3 b	2.6 a	1.4 a

[z] Seedling emergence was recorded 4 weeks after scale bulblets and one-year-old bulbs were planted in the field

[y] The recovery percentage and circumference of one-year-old bulblets and two-year-old bulbs were recorded 6 months after scale bulblets and one-year-old bulbs were planted in the field

[x] Bulblet rot index of one-year-old bulblets and two-year-old bulbs were recorded 6 months after scale bulblets and one-year-old bulbs were planted in the field based on disease rating scale of 0–5, where 0 = healthy and 5 = completely decayed. The disease severity index data (mean of the two experiments) were arcsine transformed prior to analysis

[w] Means within each column followed by the same letter are not significantly different ($P>0.05$) according to Tukey's multiple range test

[v] Scale bulblets and one-year-old bulbs treated with distilled water were used as controls

The mechanism by which the bacterial cells were killed following application of a high concentration of Sporgon remains unknown and thus warrants further investigation.

Production of scale bulbs and yearling bulbs of lily in soilless media such as BVB No.4 has become increasingly popular for the greenhouse industry in Taiwan. However, one of the most common problems of using this commercial medium BVB No.4 for growing lily is the contamination of the growth substrate by *F. proliferatum*, causing bulb rot of lily (Wu et al. 1998). The large-scale commercial greenhouse trial in this study using BVB No.4 medium indicates that scale bulbs of lily treated with Sporgon (<100 μg/mL) in combination with either *S. misionensis* strain PMS101 or *B. thermoglucosidasius* strain PMB207 (Table 2) effectively reduced severity of Furarium seedling blight of lily. This result suggests the potential of the combined treatment of Sporgon (<100 μg/mL) and microbial agents for application in the commercial production of lily bulbs in soilless culture.

While both strains of *B. thermoglucosidasius* PMB207 and *S. misionensis* PMS101 significantly reduced the incidence of Fusarium basal rot of lily grown in artificially infested field soil (Fig. 2), *B. thermoglucosidasius* strain PMB207 showed only limited effect on the control of this disease in one-year-old lily bulbs under field conditions (Table 3). One potential explanation for this could be reduced antibiotic production by *B. thermoglucosidasius* in the field. Antibiosis is a well-known mechanism for effective suppression of fungal plant pathogens by *Bacillus* spp. For example, antibiosis is involved in the control of *Botrytis cinerea*, *Rhizoctonia solani*, *Aspergillus flavus* and *Pythium aphanidermatum* by *B. subtilis* (Toure et al. 2004; Stein 2005; Leclere et al. 2005); in the control of *Phytophthora medicaginis* and *P. aphanidermatum* by *B. cereus* (Smith et al. 1993; Milner et al. 1996; Emmert et al. 2004); and in the control of *Fusarium oxysporum* and *B. cinerea* by *B. amyloliquefaciens* (Paulitz and Belanger 2001). However, numerous reports also indicate that production of antibiotics by *Bacillus* spp. can be affected by soil types (such as clay soil) (Campbell 1983; Campbell and Ephgrave 1983), organic matter (such as crop residues) (Weinhold and Bowman 1968), plant root exudates (such as carbohydrates and amino acids) (Bacilio-Jiménez et al. 2003) and minerals (such as phosphate) (Milner et al. 1995 and 1996). Besides production of antibiotics, colonization of plant roots by microbial agents (Reva et al. 2004), formation of biofilm on plant roots (Bais et al. 2004), and competition for growth space and nutrients (Handelsman and Stabb 1996) are other important factors affecting efficacy of control of plant pathogens by *Bacillus* spp. The strong antagonistic effect of *B. thermoglucosidasius* strain PMB207 against *F. oxysporum* f. sp. *lilii* in dual cultures (data not shown) suggests release of antibiotics by this antagonist. However, the reduced efficacy of *B. thermoglucosidasius* strain PMB207 for the control of disease in one-year-old lily bulbs in the field (Table 3) could be due to factors other than reduced antibiotic production, such as poor survival of the organism under natural field conditions. Therefore, more studies are required on the factors affecting efficacy of control of Fusarium diseases of lily by *B. thermoglucosidasius* strain PMB207 and *S. misionensis* strain PMS101 in natural soils and in soilless culture.

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