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Paenibacillus elgii SD17 as a Biocontrol Agent Against Soil-borne Turf Diseases

Dal-Soo Kim[1]*, Cheol-Yong Bae[1], Sam-Jae Chun[1], Do-Hyung Kim[1], Sung-Won Choi[2] and Kee-Hyun Choi[2]

[1]*LG Life Sciences Ltd./R&D Park, Daejeon 305-380, Korea*

[2]*Green Biotech Ltd., Paju-City 413-830, Korea*

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Paenibacillus elgii* SD17 (KCTC 10016BP^T = NBRC 100335^T) was recently reported as a new species. Based on its inhibitory activity to *Thanatephorus cucumeris* AG1-1, strain SD17 was further evaluated for its potential as a biocontrol agent against soil-borne diseases of turf grasses in Korea. *P. elgii* SD17 showed a broad spectrum of antimicrobial activity *in vitro* test and suppressed development of turf grass diseases; Pythium blight caused by *Pythium aphanidermatum* and brown patch caused by *T. cucumeris* AG1-1 on creeping bentgrass (*Agrostis palustris*) in the growth chamber tests. Under a condition for massive culture in a 5,000 L fermenter, *P. elgii* SD17 reached 6.4×10^8 spores/ml that resulted in approximately 1.0×10^7 cfu/g when formulated into a granule formulation (GR) using the whole culture broth instead of water. Using the GR formulation, biocontrol activity of *P. elgii* SD17 was confirmed. In the growth chamber tests, the GR formulation was effective against brown patch and Pythium blight with similar level of disease severity compared to each of the standard fungicides at the application rates of 10 g/m^2 or above. In the field tests, compared to each untreated control, the GR formulation also effectively controlled Pythium blight, brown patch and large patch at all the application rates of 5, 10 and 20 g/m^2, respectively, without significant response by the application rates. However its performance was inferior to each of the standard chemical fungicides. Based on these results, we consider this GR formulation of *P. elgii* SD17 as an effective biocontol agent to suppress Pythium blight, brown patch and large patch of turf grasses in Korea.

Keywords : biofungicide, biological control, *Paenibacillus elgii*, soil-borne diseases

In Korea, Pythium blight caused by *Pythium* spp. (Kim, 1999), brown patch caused by *Thanatephorus cucumeris* AG1-1 (Chung and Chung, 1998) on creeping bentgrass (*Agrostis palustris* cv. Pencross) and large patch caused by *T. cucumeris* AG2-2 on zoysiagrass (*Zoysia japonica*) (Shim et al., 1994) are important soil-borne diseases of turf grasses. Although these diseases have been mainly managed by applying chemical fungicides, there has been an increasing demand and trend to reduce the chemical fungicides for human safety and environmental concerns. Biological control of soil-borne diseases has been a major alternative to chemical fungicides, and many bacterial biocontrol agents have been found to inhibit the turf pathogens (Park et al., 1995). Of these bacteria, endospore-formers including *Bacillus* and *Paenibacillus* spp. are generally known to have advantages of prevalent presence in soils, tolerance to thermal stress, rapid growth in liquid fermentation, feasibility for formulation, and high survival rate for long period during storage. These characteristics have been consistently attracted for practical development and application as biocontrol agents. For example, *Bacillus cereus* (Emmert and Handelsman, 1999) and *B. subtilis* (Brannen and Kenney, 1997; Tunner and Backman, 1991) have been successfully developed as natural biocontrol and plant-growth-promoting bacteria when inoculated directly into soils as seed treatments. In Korea, there have been also several recent reports that *B. subtilis* S1-0210 is effective against *Botrytis cinerea* in strawberries (Hang et al., 2005) and *B. pumilus* B1141 and *P. lentimobus* B1146 against *Cylindrocarpon destructans* and *Phytophthora cactorum* in ginseng (Bae et al., 2004). In this study, we have used *Paenibacillus elgii* SD17 that was recently reported as a new species with descriptions on its specific morphological, cultural, biochemical and molecular characteristics (Kim et al., 2004). This study was conducted to evaluate its potential as a biocontrol agent by (1) characterizing its antimicrobial spectrum and activity, (2) optimizing its fermentation in small and large scale, (3) examining feasibility for a granule type of formulation of the fermented culture, and (4) assessing its biocontrol activity against three diseases of turf grasses in a growth chamber and fields.

Materials and Methods

***P. elgii* SD17 and pathogens.** *P. elgii* SD17 (KCTC 10016BP^T = NBRC 100335^T) was isolated from roots of *Perilla*

*Corresponding author.
Phone) +82-42-866-2336, FAX) +82-42-863-0239
E-mail) dalskim@lgls.co.kr

frutescens collected from Seocheon (Kim et al., 2004). *P. elgii* SD17 was routinely grown on one tenth strength of tryptic soy broth (1/10 TSB, Difco Laboratories, Detroit, USA) or agar (1/10 TSA) and maintained in 20% glycerol at −80°C for long-term storage. The pathogenic isolates of *Pythium aphanidermatum* P34-1, *T. cucumeris* AG1-1 S and *T. cucumeris* AG2-2 RSL-5 were kindly obtained from Korea Turfgrass Research Institute (KTRI) and routinely grown on potato dextrose agar (PDA, Difco) at 25°C for 3 days for each use. These isolates were maintained in sterile distilled water at room temperature for storage. The other fungal and bacterial isolates were used from the internal collections of LG Life Sciences, Ltd..

Antimicrobial activity *in vitro* test. Antimicrobial activity of *P. elgii* SD17 was tested against 20 fungal and 6 bacterial isolates. For antifungal activity, each fungal isolate was grown at the center on PDA, and 10 μl of the overnight culture suspension of *P. elgii* SD17 was dropped at opposite margins on the same plate. The plates were incubated at 30°C and assessed for inhibitory activity by measuring size (mm) of clear zone between *P. elgii* SD17 and each fungus at 5 days after incubation. For antibacterial activity, each bacterial isolate was grown overnight in 1 ml of Luria-Bertani (LB) broth (Difco) at 30°C and mixed with 9 ml of 1% top-layer water agar. The mixed top-layer agar was additionally poured onto LB agar and solidified inside a clean hood. The culture suspension of *P. elgii* SD17 was dropped on LB plate as described for the antifungal activity, incubated at 30°C and assessed for inhibitory activity by measuring size (mm) of clear zone between *P. elgii* SD17 and each bacterium at 3 days after incubation.

Fermentation. Several factors affecting growth and sporulation of *P. elgii* SD17 were independently studied using LB broth as the basis medium in the order of temperature, pH, carbon, nitrogen and mineral sources; the temperatures were at 20, 25, 30 and 35°C, pH at 5.0, 6.0, 7.0, 8.0 and 9.0, carbon sources as sucrose, soluble starch, glycerol, glucose, lactose, maltose and fructose, and nitrogen sources as peptone, tryptone, soybean meal, gelatin, glutamic acid, urea, ammonium sulfate, corn steep liquor and yeast extract, and minerals as magnesium sulfate, sodium chloride, calcium chloride, manganese chloride, potassium phosphate and copper sulfate. After selecting optimum temperature at 30°C and pH 7.0 in 100 ml of LB broth, each carbon, nitrogen and mineral source was added at 1% by volume in 100 ml of LB broth used as the basis medium. After selecting optimum condition of each factor, Plakettt-Burman Design (PBD) and Box-Behnken Design (Diane and Douglas, 2001) were further applied to optimize the growth and sporulation of *P. elgii* SD17 in combination of each factor in 2 L of the media in a 5 L fermenter (KF-5L, Kobiotech Co. Ltd., Korea). Bacterial growth was measured 2 days after incubation by colony forming units (cfu/ml) after dilution plating, and sporulation by spore numbers (spore/ml) using a phase microscope (Axioplan 2, Zeiss, Oberkochen, Germany). After these optimizations for temperature, pH and medium composition, respectively, *P. elgii* SD17 was grown in the optimized medium at 30°C and 80 rpm along with acidity adjusted to be at pH 7.0 or above in a 5,000 L scale of fermenter with the working volume approximately at 2,500 L per batch and monitored for its growth and sporulation.

Granule formulation. Using the broth culture from the large scale of fermentation, a GR formulation was prepared by the extrusion method commonly and commercially practiced to produce water dispersible formulation of chemical pesticides. Additives for the GR formulation were thoroughly mixed with the ratios of talc (70%), bentonite (25%), dextrin (3%) and yeast extract (2%) by weight. The bacterial culture at 20% of the additive mixture by weight was added and evenly battered before being passed through a pilot extrusion granulator (Model GR-01, Daesung Chemical Machinery Co., Korea), and dried with air stream. The GR formulation was dilution-plated on 1/10 TSA for enumeration and also kept at room temperature inside building for 6 months for stability test.

Biocontrol activity test in a growth chamber. Pathogens of Pythium blight (*P. aphanidermatum* 34-1) and brown patch (*T. cucumeris* AG1-1 S) were grown on PDA at 25°C for 7 days, and each fungal disk (12 mm in diameter) was used as innocula. Turf mats of creeping bentgrass (cv. Pencross) were purchased from a supplier (LGREEN, Gwacheon, Korea), transplanted on 2 kg of an organic soil (BuNong® Horticultural Soil No. 5, Gyeongju, Korea) evenly filled in a rectangular tray (40 cm long × 30 cm wide × 7 cm deep) and grown inside glass house for 2-3 weeks. For inoculation with each pathogen, four holes (12 mm wide, 20 mm deep) with a cork borer were evenly punctured on bentgrass mat. Two disks of each pathogen were placed into each hole and covered with plug. The GR formulation at the rates of 5, 10 and 20 g/m^2, respectively, was evenly applied by hand on the top of the bentgrass lawn inoculated with each pathogen. The broth culture of *P. elgii* SD17 that was adjusted to concentrations of 1×10^8, 1×10^6 and 1×10^4 cfu/ml, respectively, into tap water was drenched at the rate of 1.0 L/m^2 onto the tray as a reference. Chemical fungicides registered on turf grasses in Korea were used as standards as directed by manufacturers; Ridomil™ (25% WP formulation of metalaxyl, Syngenta Korea Ltd., Korea) at 1.0 L/m^2 with 2,000-fold dilution in

water for Pythium blight and Thiophan™ (70% WP of thiophante-methyl, Dongbu Jeongmil Ltd., Korea) at 1.0 L/m^2 with 5,385-fold dilution for brown patch. The treated trays were placed at 25°C for 28 days in the growth chamber (80% RH, 12 h photoperiod per day). Disease severity was visually assessed at 21 days after application on the basis of percent size with typical symptom of each disease compared to the total size of the bentgrass lawn in the tray. The experiment was designed by completely random design with three replicates. Statistical analysis (Genstat 5, Genstat Committee, Oxford University, UK) was conducted independently at each assessment for significance between treatments by analysis of variance (ANOVA) and then protected least significant difference (LSD) at P=0.05 for multiple comparisons.

Field tests. Field tests were conducted for Pythium blight, brown patch on a bentgrass nursery and additionally for large patch on a field of zoysia grasses at both nurseries of LG Life Sciences Ltd./R&D Park (Site 1) and Yuseong Country Club (Site 2) located in Daejeon, Korea in 2001 and 2002. Innocula of Pythium blight and brown patch were prepared as described in the growth chamber tests. For large patch, the innoculum was prepared by growing *T. cucumeris* AG2-2 (RSL-5) onto the sand-oatmeal medium (760 g sand, 40 oatmeal, 76 ml distilled water) for 1 month as described by Shim et al. (1994) to secure disease development under natural field condition. Each plot size was 2 m × 1 m separated by 1-m wide border between plots. Nine holes (1.2 cm in diameter, 3 cm in depth) were evenly punctured for each plot, and each innoculum preparation was placed into holes; five agar disks (12 mm in diameter) for *P. aphanidermatum* P34-1 and *T. cucumeris* AG1-1 S and 2 g of the sand-oatmeal medium for *T. cucumeris* AG2-2. The granule formulation was applied at two rates of 10 g/m^2 and 20 g/m^2, respectively, based on results from the growth chamber. After inoculation of each pathogen and application with the GR formulation, sufficient irrigation was made on each field with water sprinkler. Further irrigations were periodically made twice a day during the tests. Chemical fungicides registered on turf grasses in Korea have been used as the commercial standards. In addition to Ridomil™ and Thiophan™, Pencycuron™ (25% WP of pencyruon, Bayer Crop Science Korea Ltd., Korea) was applied at 1.0 L/m^2 with 1,000-fold dilution for large patch. Each treatment was applied four times with 7-10 days of interval. Randomized complete block design was used with three replicates for each field test. Disease severity was visually assessed at 7 days after the last application on the basis of percent size with typical symptom of each disease compared to each of total plot size. Statistical analysis was made same for the growth chamber test as described above.

Results and Discussion

Antimicrobial activity. *P. elgii* SD17 showed a broad antimicrobial activity against all 20 fungal and 6 bacterial isolates tested in this study (Table 1). The target pathogens, *P. aphanidermatum*, *T. cucumeris* AG1-1 S and AG2-2 RSL-5 in this study for the biological control, were also inhibited by *P. elgii* SD17. The other fungal isolates including plant pathogens and a saprophyte, *Trichoderma viridae*, were also inhibited. Two bacterial isolates, *Escherichia coli* DH5α and *Bacillus subtilis* were also sensitive to *P. elgii* SD17. It is noteworthy that *P. elgii* SD17has a broad antimicrobial spectrum against all the microorganisms tested, regardless of their taxonomic position. This may be due to secondary metabolites produced by *P. elgii* SD17 during growth like other bacilli. In our preliminary studies, *P. elgii* SD17 appeared to produce depsipeptide antibiotics of permetin A family that is report-

Table 1. Antimicrobial activity of *P. elgii* SD17 *in vitro* test

Microorganism[a]	Inhibition width (mm)[b]
Fungi	
Cladosporium resinae	6.0
Fusarium oxysporum f. sp. *lycopersici*	5.7
Chaetomium globosum	5.3
Sclerotinia homoecocarpa	5.0
Phytophthora infestans	4.3
Magnaporthe grisea KJ401	4.0
Corynespora cassicola	3.7
M. grisea KJ201	3.3
Pythium aphanidermatum P34-1	3.3
Botrytis cinerea BC2	3.0
T. cucumeris AG1	2.7
Colletotrichum gloeosporioides	2.3
T. cucumeris AG1-1 S	2.3
T. cucumeris AG2-2 RSL-5	2.0
C. gloeosporioides CalC-19	2.0
Rhizopus sp.	2.0
Trichoderma viride 6047	2.0
C. gloeosporioides CaKJ-12	1.3
C. gloeosporioides CaKJ-4	1.0
T. cucumeris AG4	0.5
Bacteria	
Pseudomonas fluorescens	5.0
Xanthomonas oryzae	4.3
X. vesicatoria	4.1
Escherichia coli DH5α	3.4
Bacillus subtilis	2.1
Burkholderia glumea	1.0

[a] Fungal isolates were incubated on PDA at 25°C for 5 days, and bacterial isolates grown on LB agar at 30°C for 3 days.
[b] The inhibition widths were determined by measuring clear zone between *P. elgii* SD17 and each fungus or bacterium.

Table 2. Effect of temperature and pH on the growth and sporulation of *P. elgii* SD17

Factor		Growth[a] (cfu/ml)	
		Vegetative	Spore
Temperature	20°C	3.0×10^6	3.7×10^5
	25°C	6.0×10^7	9.0×10^5
	30°C	3.4×10^8	3.5×10^7
	35°C	1.0×10^8	1.4×10^7
	37°C	8.9×10^7	1.6×10^6
pH	5.0	6.5×10^4	3.9×10^3
	6.0	8.7×10^7	5.4×10^5
	7.0	3.5×10^8	3.6×10^7
	8.0	1.2×10^8	2.8×10^7
	9.0	7.2×10^6	4.3×10^5

[a] Growth was measured for vegetative cells by counting colony forming units (cfu) after dilution plating, and for spores by counting spore numbers (spore/ml) using a phase microscope.

ed by isolates of *B. circulans* with a broad antimicrobial activity against Gram-positive and -negative bacteria (Sugawara et al., 1984; Takahara et al., 1979). Identification of molecular structure on these metabolites is currently in progress.

Fermentation and formulation. Of the factors tested in this study, the optimal growth conditions were 30°C for temperature and 7.0 for pH (Table 2). Based on the results of nutrient sources (Table 3), each nutrient was further optimized by the PBD method and selected for massive culture in 1.0% of soluble starch as carbon source, 1.0% of yeast extract and 1.5% of soybean meal as nitrogen source and 0.15% of magnesium sulfate as mineral source. Bacterial growth and sporulation were monitored along with pH in the massive culture (Fig. 1). Population of *P. elgii* SD17 reached to nearly 4.3×10^9 cfu/ml near at 24 h after fermentation, decreased to nearly 1.0×10^9 cfu/ml around 54 h and stabilized thereafter. The endspores were observed after the culture for 30 h, and the spore numbers slightly increased over time and reached approximately 6.4×10^8 cfu/ml at 54 h that is 14.9% of the viable cells.

The GR formulation was produced without any critical technical problem during the formulation process that has resulted in the viable cells approximately at 1.1×10^7 cfu/g that is 1.6% of the endospores. *P. elgii* SD17 in the GR formulation was turned out to be stable at least for 6 months when stored at room temperature. Considering the low sporulation and survival rate, further efforts are still needed to improve the sporulation rate in order to minimize the reduction of viable cells after formulation. Regarding formulation of biocontrol agents, Oh et al. (2005) reported that a GR formulation with *Paenibacillus* sp. AC-1 was stable up to 12 weeks after storage at the wide storage temperature (4-50°C). This GR formulation was comprised of 20% of *Paenibacillus* sp. AC-1 powder, 75% of polycarboxylate as surface-active agent, 1% of sodium poly-

Table 3. Effect of sources of carbon, nitrogen and mineral on the growth and sporulation of *P. elgii* SD17

Nutrient		Growth[a] (cfu/ml)	
		Vegetative	Spore
Carbon	Control	3.4×10^8	3.6×10^7
	Sucrose	2.0×10^8	1.2×10^7
	Soluble starch	5.5×10^8	5.0×10^7
	Glycerol	7.2×10^7	3.4×10^6
	Glucose	1.4×10^8	1.3×10^7
	Lactose	2.3×10^8	1.4×10^7
	Maltose	1.2×10^8	2.3×10^7
	Fructose	8.7×10^7	3.7×10^6
Nitrogen	Control	5.4×10^8	5.1×10^7
	Peptone	7.8×10^7	8.7×10^7
	Tryptone	1.2×10^8	2.1×10^7
	Soybean meal	7.2×10^8	6.5×10^7
	Gelatin	4.2×10^8	1.3×10^7
	Glutamic acid	5.2×10^8	1.4×10^7
	Urea	4.7×10^8	2.3×10^7
	Ammonium sulfate	4.2×10^8	7.5×10^7
	Corn steep liquor	6.0×10^8	3.2×10^7
	Yeast extract	7.3×10^8	6.6×10^7
Mineral	Control	2.6×10^9	2.5×10^8
	Magnesium sulfate	4.4×10^9	3.1×10^8
	Sodium chloride	1.2×10^9	1.1×10^8
	Calcium chloride	1.3×10^9	1.2×10^8
	Manganese chloride	1.1×10^9	1.2×10^8
	Potassium sulfate	2.1×10^9	3.0×10^8
	Copper sulfate	1.8×10^9	3.0×10^8

[a] Growth was measured for vegetative cells by counting colony forming units (cfu) after dilution plating, and for spores by counting spore numbers (spore/ml) using a phase microscope.

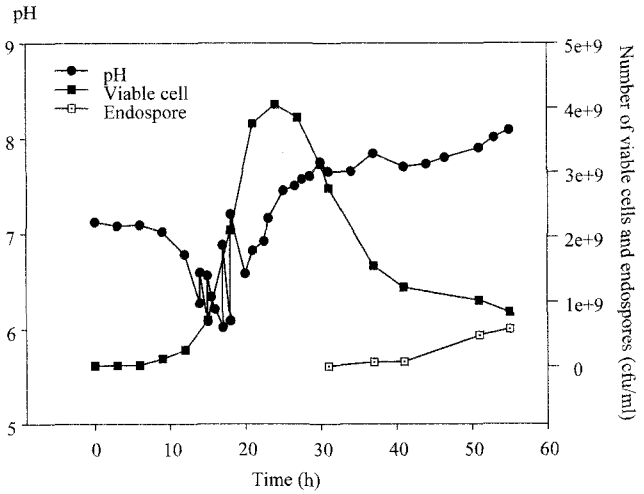

Fig. 1. Population dynamics of viable cells and endospores of *P. elgii* SD17 along with the pH changes in a 5,000 L fermenter.

acrylate as adjuvant and the rest as carrier, whereas the GR formulation for *P. elgii* SD17 consists of talc, bentonite, dextrin and yeast extract. It is interesting to notice that recipes of formulations are specified in details that may lead to comparison between biocontrol agents.

Growth chamber tests. The fermented broth and the GR formulation of *P. elgii* SD17 showed effective biocontrol activity. For Pythium blight, the disease severities treated with the fermented broth were 5.0% and 8.3% at 10^{11} and 10^{9} cfu/m^2, respectively, that were equivalent to the chemical standard metalaxyl (Table 4). The disease severity at 10^{7} cfu/m^2 significantly increased up to 28.3% but still suppressed the disease development compared to the control showing 53.3% of disease severity. The GR formulation also showed similar activity at the highest rate (20 g/m^2) with 10.0% of disease severity, although its initial population at 2.0×10^{8} cfu/m^2 is much lower than the fermented broth at 1.0×10^{11} cfu/m^2. For brown patch, disease severities were 6.7% for the fermented broth at the highest dose, 11.7% for the GR formulation, and 6.7% for the chemical standard thiophanate-methyl without significant difference. Considering that the application rate for the fermented broth and the GR formulation, *P. elgii* SD17 is needed approximately at 10^{8} cfu/m^2 for effective suppression of disease development. This population density may be needed for its initial settlement in soil and rhizosphere after application and before inoculation of each pathogen. Further studies are needed to monitor the population of *P. elgii* SD17 in soil after application to correlate the disease control activity with its population in soil.

Table 4. Biocontrol activity of *P. elgii* SD17 against Pythium blight and brown patch of turf grasses in the growth chamber tests

Treatment and application rate	Disease severity (%)[a]	
	Pythium blight	Brown patch
Control	53.3 A[b]	33.3 A[b]
Culture broth		
1×10^{11} cfu/m^2	5.0 C	6.7 C
1×10^{9} cfu/m^2	8.3 C	11.7 BC
1×10^{7} cfu/m^2	28.3 B	30 A
GR formulation		
2×10^{8} cfu/m^2 (= 20 g/m^2)	10.0 C	11.7 BC
1×10^{8} cfu/m^2 (= 10 g/m^2)	13.3 C	13.3 BC
5×10^{7} cfu/m^2 (= 5 g/m^2)	26.7 B	15.0 B
Chemical fungicide[b]		
Metalaxyl at 250 mg ai/m^2	5.0 C	–
Thiophanate at 250 mg ai/m^2	–	6.7 C

[a] Disease severity was visually assessed at 7 days after the last application on the basis of percent size with typical symptom of each disease compared to each of total plot size.
[b] Means in each column with the same letter are not significantly different by protected LSD at P = 0.05.

Field tests. The biocontrol activity of *P. elgii* SD17 in the growth chamber tests was confirmed in the six field tests conducted at the two nursery sites of LG Life Sciences Ltd./R&D Park and Yuseong Country Club in Daejeon, Korea in 2001 and 2002. Similar trends of biocontrol activity of the GR formulation for Pythium blight and brown patch were observed for Pythium blight and brown patch as observed in the growth chamber tests (Table 5). In the field tests, its biocontrol activity was included for large patch mainly due

Table 5. Biocontrol activity of *P. elgii* SD17 against Pythium blight, brown patch and large patch of turf grasses in the field tests

Treament	Disease severity (%)[a]					
	Pythium blight		Brown patch		Large patch	
	Site[b] 1	Site 2	Site 1	Site 2	Site 1	Site 2
Control	28.3 A[c]	46.7 A	27.3 A	33.3 A	20.0 A	18.3 A
GR formulation						
2×10^{8} cfu/m^2 (= 20 g/m^2)	8.3 B	13.3 C	8.3 B	11.7 B	5.0 C	6.7 B
1×10^{8} cfu/m^2 (= 10 g/m^2)	10.0 B	15.0 C	10.3 B	13.3 B	5.0 C	6.3 B
5×10^{7} cfu/m^2 (= 5 g/m^2)	10.7 B	23.3 B	14.7 B	20.0 B	11.7 B	10.0 B
Chemical fungicide						
Metalaxyl at 250 mg ai/	5.7 B	8.3 CD	–	–	–	–
Thiophanate at 200 mg ai/	–	–	4.7 C	5.0 C	–	–
Pencycuron at 250 mg ai/	–	–	–	–	3.3 C	4.0 C

[a] Disease severity was visually assessed at 7 days after the last application on the basis of percent size with typical symptom of each disease compared to each of total plot size.
[b] Site 1 is located at LG Life Sciences/R&D Park and site 2 at Yuseong Country Club.
[c] Means in each column with the same letter are not significantly different by protected LSD at P = 0.05.

observed for Pythium blight and brown patch as observed in the growth chamber tests (Table 5). In the field tests, its biocontrol activity was included for large patch mainly due to its practical importance of disease management in turf grasses and convenient visual assessment based on large patches formed in field. The GR formulation was also effective in suppressing development of large patch (Table 5). We have not observed any significant differences between the two sites for each disease. However, in the fields, overall performance of the GR formulation inferior to that in the growth chamber even at the highest rate of 20 g/m^2 whereas the chemical standards reasonably maintain the control activity. Griffiths et al. (1999) reported that microbial community structure consistently changes with dependence on substrates added in soil. Frey et al. (1999) also reported that bacterial and fungal biomass were significantly affected by water content in soil and organic substrates. These observations indicate that environmental conditions in soil or on soil surface of turf grasses may have negatively affected vitality of *P. elgii* SD17 in natural conditions with direct sunlight or limited substrates dependent on water content available for its growth. As in the growth chamber test, a similar weak dose response for the GR formulation was also observed through the tests. This observation may be explained by that the total number of *P. elgii* SD17 per unit area for the GR formulation is smaller mainly due to the lower dilution effect for each treatment and three times of the weekly applications during the field tests. This indicates that protection or maintenance of biocontrol agent after application *in situ* is another important factor to be considered in development of biocontrol agent especially by optimizing the formulation.

Based on the results, we have found that *P. elgii* SD17 has a very broad antimicrobial spectrum that is rather unusual and reaches up to 4.3×10^9 cfu/ml as the highest population. We were able to produce the GR formulation at least to the quality of the chemical pesticides without any critical difficulties. Furthermore, the GR formulation effectively controls the three soil-borne diseases of turf grasses in the growth chamber and field tests. Therefore, we propose *P. elgii* SD17 in the GR formulation as a potential biocontrol agent for practical application, although there are still several tasks to be improved for higher population and sporulation during culture and further optimization of formulation for efficient establishment in soil.

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