

Effects of *Streptomyces griseofuscus* 200401 on Melon Powdery Mildew in Greenhouse

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The fermentation broth (FB) of *Streptomyces griseofuscus* 200401 isolated from non-farming soil showed antagonistic activity against powdery mildew fungus both in melon leaf/seedling assay and in field trials. The FB of *S. griseofuscus* 200401 was tested at different concentrations. In primary test, the control value of 2-fold diluted FB of *S. griseofuscus* 200401, compared to control, reached to 82.8%. The protective activity recorded 80.5% in 2-fold dilution of FB. The effect was reduced to 28.9% in high dilution (100-fold) treatment. The curative effect was relatively lower than protective activity. In field test, the antifungal activities of *S. griseofuscus* 200401 remained low in blocks sprayed with 100-fold diluted FB and the control values were 15.2 and 15.9% in 2005 and 2006, respectively. However, the activities were as high as 65% and 67.3% in the blocks treated with 2-fold dilution of FB during the same period.

Keywords : antifungal activity, melon, powdery mildew, *Streptomyces griseofuscus*

Most of the greenhouse plants such as melon, strawberry, rose, and cucumber suffered from powdery mildew (PM) caused by *Sphaerotheca aphansis*, *S. fuliginea* and *S. pannosa* var *rosae* (Choi et al., 2004; Elad et al., 1996).

The control of PM is achieved by repeated foliar applications of chemical fungicides such as azoxystrobin, bitertanol, fenarimol, and hexaconazole (Cho et al., 2005; Karaoglanidi and Karadimos, 2006). However, chemical control led to disadvantages such as environmental pollution, phytotoxicity, and development of fungicide-resistant strains to fungicides (Elad et al., 1996; Lim et al., 2006). In order to minimize these problems, researchers have developed eco-friendly alternative management strate-

gies including use of biocontrol agents, mineral salt, essential oil, and plant extracts for the management of plant diseases (Elad et al., 1996; Fernandez et al., 2006; Rémus-Borel et al., 2005; Schuerger and Hammer, 2003; Suárez-Estrella et al., 2007; Zang et al., 2005).

Choi et al. (2004) reported that chrysophanol, parietin, and nepodin isolated from *Rumex crispus* are effective to barely powdery mildew caused by *Blumeria graminis* f. sp. *hordei*. Si (Silicon) induced systemic resistance on a number of plants on soil and hydroponic cultivation through a lot of mechanisms including biosynthesis of phenolic and fungitoxic-compound (Rémus-Borel et al., 2005; Schuerger and Hammer, 2003). Biocontrol agent mixed with *Verticillium lecanii*, *Bacillus subtilis*, and *Tilletiopsis minor* showed high control efficacy against mango powdery mildew in Egypt (Nofal and Haggag, 2006).

In this study, the disease control activity to melon powdery mildew by *Streptomyces griseofuscus* 200401 isolated from forest soil, which showed antifungal activity against *Phytophthora capsici* and *Colletotrichum acutatum* (Lim, 2005), was investigated on melon seedlings grown in greenhouse.

Materials and Methods

Antifungal microorganism. An actinomycete strain, *S. griseofuscus* 200401, was isolated from forest soil of Gabjongsan mountain in Sangju-city, Korea (Lim, 2005). It was maintained at -50°C in yeast malt extract broth (YMB) containing 3 g of yeast extract, 3 g of malt extract, 5 g of peptone, 10 g of dextrose, and 1 liter of 20% glycerol distilled water. For the experiment, the actinomycete strain was grown on yeast malt extract agar (YMA) for 7 days at 28°C. To evaluate the control efficacy, the antagonist was inoculated in 1,000 ml Erlenmeyer flasks containing 500 ml of YMB and then shook it at 150 rpm for 7 days at 28°C.

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The concentration of the antagonist was adjusted to 10^6 cfu/ml and 10^7 cfu/ml by using a standard curve on the spectrophotometer.

Primary test. Leaves of 2 weeks old melon plants (cv. Kumssaragi, Hungnong, Korea) grown in greenhouse were placed on wet filter papers in Petri dishes. Inoculums were prepared by gently brushing fresh powdery mildew colonies on naturally infected melon leaves in 100 ml of 0.01% Tween 20. In order to test efficacy of *S. griseofuscus* 20041, its fermentation broth (FB) was mixed with conidial suspension (3×10^5 conidia/ml) of a powdery mildew fungus. The concentration of FB with cells of *S. griseofuscus* 20041 in suspension mixture was adjusted to 1% (10^6 cfu/ml), 10% (10^7 cfu/ml), and 50% (5×10^7 cfu/ml). The mixture (5 μ l) of the antagonist and the pathogen was dropped at every twenty points on a leaf. Leaves inoculated with 50 μ l of conidial suspension (3×10^5 conidia/ml) or with sterile water were used as positive- and negative-control, respectively. The dishes of treated melon leaves were incubated at $25 \pm 2^\circ\text{C}$ for 24 hours in darkness to induce spore germination and followed by a photoperiod of 12 hours of dark and 12 hours of light cycle schedule. Symptoms of powdery mildew on inoculated leaves were checked 4 days after incubation. Six leaves were inoculated and three replications were maintained in each treatment.

Protective and curative effects of the antagonist on melon powdery mildew were tested on melon seedlings. To test protective effect, diluted FBs of *S. griseofuscus* 20041 by 2- and 100-fold were sprayed twice on seedling leaves at 7 days interval. Then the leaves were inoculated with powdery mildew suspension of 3×10^5 conidia/ml on 24 hours after the second spray of FBs of the actinomycete strain. For a test of curative effect, 2- and 100-fold dilution of the FB of *S. griseofuscus* 20041 were applied to melon leaves with a sprayer when several colonies of powdery mildew appeared on leaves. There were three leaves per replication and three replications were maintained per each treatment.

In all experiments, the control efficacy was figured up as the ratio of the numbers of colonies on treated leaves compared with the control leaves.

Field test. The experiments were conducted from 2005 to 2006 in Gyeongsan area, where serious powdery mildew problem is prevalent by *S. fuliginea* on melon cultivation, Gyeongbuk, Korea. The powdery mildew fungus-susceptible melon cultivar "Kumssaragi" was used in this experiment. Field plots consisted of three 30×1 m (length \times width) rows which were spaced 50 cm apart from each row and arranged through randomized block design with three replications. On April 22, each plot was treated

with one of FB of *S. griseofuscus* 20041, fungicide fenarimol, and distilled water (DW) depending on the design. Each solution was sprayed on leaf surface until run-off by using hand-sprayer (approximately 2 L of solution per replicate). The FB of *S. griseofuscus* 20041 was diluted with DW to 2- and 100-fold. The treatments with DW alone and the fungicide fenarimol (40 μ g a.i./ml) served as negative and positive controls, respectively. All the cultural practices other than powdery mildew control including irrigation and fertilization were carried out as per recommendations.

On April 29 and May 30 in 2005 and 2006, leaves of melon plants in each experimental plots were rated for powdery mildew. The disease severity rating was based on a 0-4 scale, where 0=no powdery mildew colony was observed on leaf, 1=1 to 9 colonies per leaf, 2=10 to 39 colonies, 3=40 to 79 colonies, and 4=more than 79 colonies per leaf.

Results and Discussion

The FB of *S. griseofuscus* 200401 isolated from non-farming soil showed high antifungal activity against *S. fuliginea* on melon leaves (Table 1). When culture suspension was added to 1% of conidial suspension of a powdery mildew fungus, as low as 12.8% of infection decreased compared to pathogen (conidia) only treatment. However, the high concentration of *S. griseofuscus* 200401 increased the antifungal activity of it. When the concentration of its FB reached to 50% of powdery mildew conidial suspension, inhibitory effect on the infection of powdery mildew increased to 82.8% (Table 1).

The protective and curative activities of *S. griseofuscus*

Table 1. Inhibitory effects of *Streptomyces griseofuscus* 200401 on the colonization of powdery mildew fungus in melon leaf assay

Treatment	No. of inoculations per leaf	No. of lesions per leaf ^x	Control value (%) ^y
Control	20	0	-
Pathogen only	20	18.7 \pm 0.23	-
Pathogen+1% FB ^z	20	16.3 \pm 1.12	12.8d
Pathogen+10% FB	20	10.2 \pm 0.15	45.5c
Pathogen+20% FB	20	5.1 \pm 1.02	72.7b
Pathogen+50% FB	20	3.2 \pm 0.25	82.8a

^xValues are shown as mean \pm standard deviation. The number of lesions on leaves was counted 4 days after inoculation.

^yControl value (%) is $\{1 - (\text{No. of colony on treatment} / \text{No. of colony on pathogen only})\} \times 100$. Different letters mean significant difference between treatments ($P < 0.05$).

^zFB represents fermentation broth of *S. griseofuscus* 200401.

Table 2. Protective effects of *S. griseofuscus* 200401 against powdery mildew fungus in melon seedling assay

Treatment	No. of colony before treatment	No. of colony after treatment ^x	Control value (%) ^y
Control	0	29.7±1.34	–
2-fold	0	5.8±1.48	80.5a
100-fold	0	21.1±2.31	28.9b

^xValues show mean±standard deviation. The numbers of colony on leaves were counted 7 days after treatment. Different letters mean significant difference between treatments ($P<0.05$).

^yControl value (%) is $\{1-(\text{No. of colony on treatment}/\text{No. of colony on control})\} \times 100$.

Table 3. Curative effect of *S. griseofuscus* 200401 against powdery mildew fungus in melon seedling assay

Treatment	No. of colony before treatment ^x	No. of colony after treatment ^y	Control value (%) ^z
Control	31.2±1.45a	65.3±1.82a	–
2-fold	29.3±2.54a	9.5±1.27c	85.5a
100-fold	35.5±3.34a	26.4±2.34b	59.8b

^{x,y}Values show mean±standard deviation. The numbers of colony on leaves were counted 7 days after treatment.

^zControl value (%) is $\{1-(\text{No. of colony on control}/\text{No. of colony on treatment})\} \times 100$.

Different letters in the columns mean significant difference between treatments ($P<0.05$).

20041 on powdery mildew were given in Tables 2 and 3. Its protective activity against powdery mildew activity was 80.5% in 2-fold dilution. However, the activity decreased with increasing dilution rate of FB; the protective activity was reduced to 28.9% when 100-fold dilution of FB was treated (Table 2). The curative effect was comparatively lower than protective activity; the curative activities were 85.5% and 59.8% with the same treatment in protective activity experiment (Table 3). In the field tests conducted in 2005 and 2006 (Fig. 1), powdery mildew caused severe problems, and the disease severity rated 44.2% (± 1.34 standard deviation) in 2005 and 38.2% (± 2.43 standard deviation) in 2006. The fungicide fenarimol showed control values of 89% and 93.1% in 2005 and 2006, respectively. The antifungal activities in 2-fold dilution of FB showed control values of 65% and 67.3% in 2005 and 2006, respectively. On the other hand, the control values in 100-fold diluted culture suspension remained as low as 15.2% and 15.9%.

All the above results revealed that *S. griseofuscus* 20041 was highly effective against the infection of powdery mildew fungus on melon. The results of protective effect and greenhouse tests suggest that application of *S. griseofuscus* 20041 during the beginning of a season, probably, reduce the density of the secondary inoculums in greenhouses (Schuerger and Hammer, 2003). No phy-

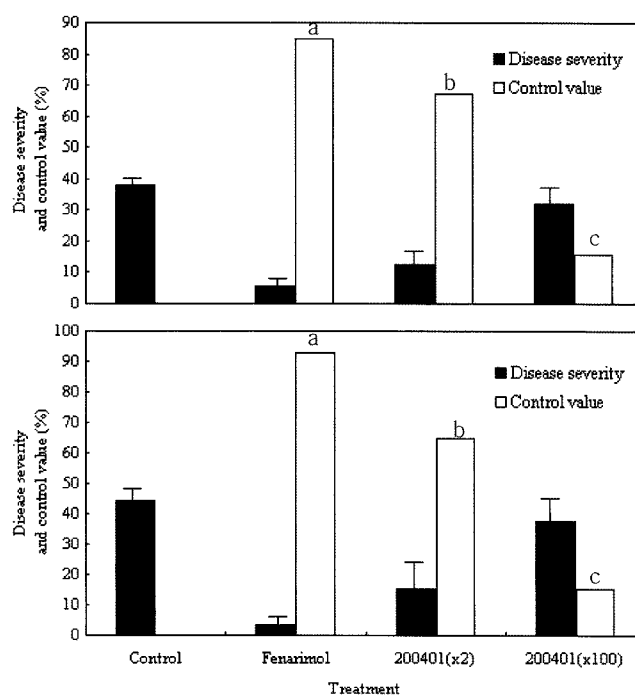


Fig. 1. Effects of *S. griseofuscus* 200401 on melon (*Cucumis melo* L.) powdery mildew in greenhouse in 2005 (bottom) and 2006 (top). Different letters and bar on the column represent significant difference between treatments ($P<0.05$) in the same year and mean±standard deviation.

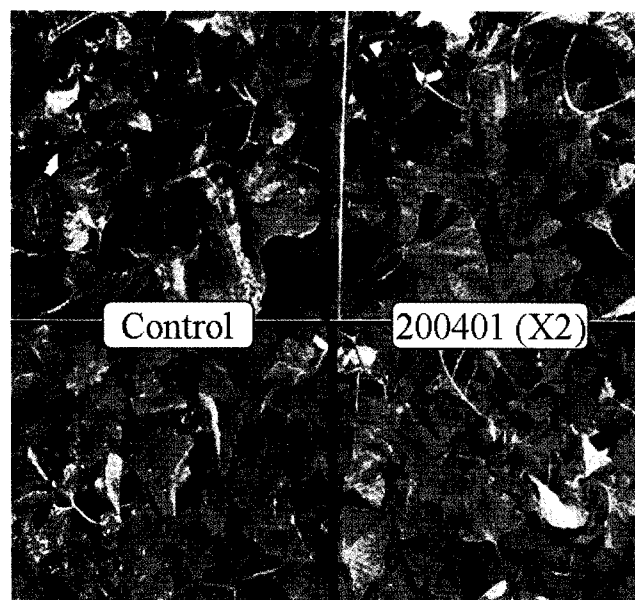


Fig. 2. Effects of *S. griseofuscus* 200401 on melon powdery mildew in greenhouse in 2005 (bottom) and 2006 (top). Left: Untreated control, Right: Treated with 2-fold diluted fermentation broth of *S. griseofuscus* 200401.

tototoxic effect appeared on any part of the melon plants even on spraying of 2-fold culture suspension in greenhouses. Presently, the most popular and frequently used

strategy for the control of powdery mildew is spraying chemical fungicides (Cho et al., 2005; Karaoglanidis and Karadimos, 2006). However, applications of chemical fungicides have several disadvantages including the development of resistance to fungicides in certain strains (Schuerger and Hammer, 2003; Zhonghua and Michailides, 2006). Especially, the resistance development of powdery mildew fungi to ergosterol biosynthesis-inhibitors and strobilurins led to reduced efficacy of these fungicides and induced serious problem (Zhonghua and Michailides, 2006). These situations forced to develop alternative methods to control powdery mildew and do researches on the selection and practical use of potential biocontrol agent.

S. griseofuscus 20041 having strong antifungal activity against *P. capsici* and *C. acutatum* (Lim, 2005) showed possibility as an agent to control of powdery mildew in this research. Based on the researches on *S. griseofuscus* 20041, it seems to have a comparatively broad antagonistic spectrum and a promising biocontrol agent for certain major diseases on crops. If several studies including safety, stability, identification of active compound, and fermentation method to enhance antagonistic activity be completed in the future, this antagonistic actinomycete strain will be useful in developing agent for the control of powdery mildew.

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