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SHORT COMMUNICATION

Biocontrol of Pepper Anthracnose by a New *Streptomyces* sp. A1022 under Greenhouse Condition

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Abstract A biocontrol agent including *Streptomyces* sp. A1022 was formulated into the form of a solid concentrate (SC) to evaluate its effect on anthracnose (*Colletotrichum gloeosporioides*) of pepper plants and was compared to that of commercial fungicide, azoxystrobin. Percent disease incidence was in the following order: untreated control > azoxystrobin > *Streptomyces* sp. A1022 SC. The average weight per pepper fruit was higher with *Streptomyces* sp. A1022 SC than those with azoxystrobin and the untreated control. The root length of the pepper plant postharvest was in the following order: *Streptomyces* sp. A1022 SC > azoxystrobin > the untreated control. Results showed that the pepper plants were more protected from anthracnose and under better growth conditions with *Streptomyces* sp. A1022 SC than with azoxystrobin and the untreated control.

Keywords anthracnose · biopesticides · *Colletotrichum gloeosporioides* · pepper · *Streptomyces*

Pepper (*Capsicum annum* L.) is one of the most important fresh market vegetables in Korea; however its yield and quality have been frequently limited by anthracnose, which is caused by the pathogen *Colletotrichum gloeosporioides* (Kim et al., 1999; Park et al., 2006). Control of anthracnose has been accomplished mainly with chemical fungicides, such as azoxystrobin, chlorothalonil, and mancozeb. Chemical control of the disease requires biweekly or monthly application of fungicides, which could be damaging to the environment, and frequent use of chemical fungicides could lead to the development of fungicide-resistant strains (Onyeka et al., 2006).

Biological control of diseases and pests of crops using microbial antagonists is considered an eco-friendly alternative to the use of chemical pesticides (Moënne-Loccoz et al., 2001) and is being extensively studied with several different plant diseases using a variety of microbial antagonists as part of integrated disease management programs. Soil actinomycetes are known producers of several secondary metabolites and have been shown to suppress a variety of pathogens (Samac et al., 2003; Minuto et al., 2006). Among the different actinomycetes, *Streptomyces* sp. has the potential to control soil-borne as well as foliar fungal pathogens in diverse plant hosts (Palaniyandi et al., 2011). However, many of these biological control agents are still being tested and not yet commercially available. Thus, there is a high demand for more diverse commercial biocontrol agents. In our previous study, a new *Streptomyces* sp. A1022 was isolated and characterized as a potential biocontrol agent (Lee et al., 2011). In the present work, the potential of using *Streptomyces* sp. A1022 to control pepper anthracnose under greenhouse conditions was examined.

The pepper seeds of Longdari 35 (AsiaSeed Co., Korea) were sown in plastic pots. Pepper seedlings were removed after 38 days and transplanted in a sandy loam soil (pH 6.2, organic matter content of 5.2%) in a greenhouse and were allowed to grow further until the end of the experiment. Fertilizers were applied in the form of organic fertilizer with N : P_2O_5 : K_2O (20 : 10 : 5) in kg/10 a. Fifty percent of the fertilizer was applied before transplanting and the remaining 50% was applied 25 days after transplantation. A randomized complete block design was used with three replicates per trial. The cultivation densities of four plants/m^2 and

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Table 1 Time table for planting, inoculation, infection, and harvest

Treatment	Date
Planting	2010. 5. 29.
Artificial inoculation	2010. 7. 05.
Trial 1: Beginning treatment with *Streptomyces* sp. A1022 solid concentration and azoxystrobin before artificial inoculation	2010. 6. 29. 2010. 7. 13. 2010. 7. 27. 2010. 8. 03. 2010. 8. 10.
Trial 2: Beginning treatment with *Streptomyces* sp. A1022 solid concentration and azoxystrobin after artificial inoculation	2010. 7. 13. 2010. 7. 27. 2010. 8. 03. 2010. 8. 10. 2010. 8. 17.
Pepper harvest date	2010. 7. 13. 2010. 7. 27. 2010. 8. 24. 2010. 9. 14. 2010. 9. 28. 2010. 10. 12.

twelve plants per replicate were used.

The commercial standard fungicide, azoxystrobin (20% solid concentrate, Syngenta Korea) was purchased from a local market. The fungicide was diluted in tap water to the concentration recommended by the manufacturers (1,000 mg/L). A semi-commercial biofungicide, including *Streptomyces* sp. A1022, was formulated as a solid concentrate (SC). First, *Streptomyces* sp. A1022 was grown in medium containing 0.75% soya flour, 0.3% yeast extract, 3.375% glucose, 0.025% $CaCO_3$, and 0.025% $MgSO_4 \cdot 7H_2O$. After cultivation, the whole broth was treated for 30 min at 60±5°C, agitation of 50–100 rpm, and dissolved oxygen of 50%. The treated broth was then ground using a colloidal miller (Sinwon Industrial Machine, Korea) so that 85% of the vegetative cells could pass through a 50-mesh screen. Glycerol (10%; w/v) and polyoxyethylene nonylphenyl ether (5%; w/v) were added to the ground broth sequentially and mixed for 10 min. The final SC was diluted by a factor of 100 in tap water to a final concentration of 1×10^7 cfu/mL before application.

Spore suspensions for artificial inoculation were prepared from 8-day-old axenic cultures of *C. gloeosporioides* by flooding with sterile distilled water and dislodging the spores with a small brush. The suspension was filtered through two layers of sterile muslin cheesecloth. The spore concentration was determined using a haemocytometer and adjusted to 1.0×10^6 spores/mL of distilled water.

The efficacy of the *Streptomyces* sp. A1022 SC under semi-commercial condition against pepper anthracnose was compared to that of a chemical pesticide. In 2010, two experimental trials (trials 1 and 2) were carried out in a greenhouse located in Yeoju, Korea. The *Streptomyces* sp. A1022 SC and azoxystrobin were sprayed before and after the artificial inoculation for trials 1 and 2. Subsequently, A1022 SC and azoxystrobin were sprayed onto pepper plants four additional times in both trials (Table 1).

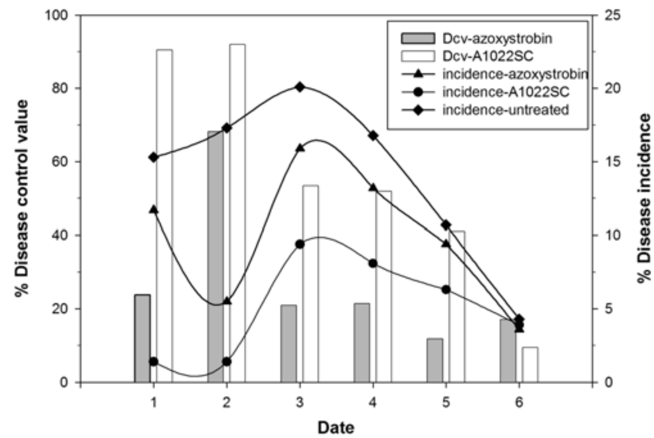

Fig. 1 Progress curves for incidence of anthracnose of pepper plants (cv. Longdari 35) with the first time spraying of *Streptomyces* sp. A1022 solid concentrate and azoxystrobin before artificial inoculation. Dates: 1, 2010. 6. 29; 2, 2010. 7. 13; 3, 2010. 7. 27; 4, 2010. 8. 3; 5, 2010. 8. 10. Percent disease control value (Dcv) = (% disease incidence with untreated - % disease incidence with treatment) / % disease incidence with untreated). A higher % disease control value indicates a lower disease incidence compared to the untreated control.

Marketable pepper fruits (over 8 cm long) were harvested six times. Fresh weights and numbers of fruits per plots were determined in all treatments. The changes in the soil microbial population (i.e., fungi, actinomycete, and bacteria) at the beginning and the end of experiment were measured as previously described (Lee et al., 2004).

Figure 1 shows the changes in the extent of disease incidence and disease control value when *Streptomyces* sp. A1022 SC and azoxystrobin were sprayed for the first time before the artificial inoculation. Throughout the whole cultivation period, % disease incidence was in the following order: the untreated control > azoxystrobin > *Streptomyces* sp. A1022 SC. The percent disease control value with *Streptomyces* sp. A1022 SC was much higher than that with azoxystrobin. These results suggest that *Streptomyces* sp. A1022 SC is very efficient in suppressing the incidence of *C. gloeosporioides*. When *Streptomyces* sp. A1022 SC and azoxystrobin were sprayed for the first time after the artificial inoculation, the % disease control value with azoxystrobin on date 1 (Fig. 2; dates: 1, 2010. 6. 29; 2, 2010. 7. 13; 3, 2010. 7. 27; 4, 2010. 8. 3; 5, 2010. 8. 10) as higher than that with *Streptomyces* sp. A1022 SC, whereas at the other dates, the trend was very similar with the above finding (Fig. 2). This result indicates that, for better initial disease control, *Streptomyces* sp. A1022 SC should be sprayed before the incidence of *C. gloeosporioides*.

Both the disease control and pepper productivity are important during the plant cultivation. Thus, peppers were harvested six times (Table 1) and the average weight per pepper fruit was obtained from the fruit numbers and fruit fresh weight (Table 2). The average weight per pepper fruit treated with *Streptomyces* sp. A1022 SC was equal to or higher than those with azoxystrobin and the untreated. This result suggests that the pepper plant is

Table 2 Fruit numbers and weight of pepper plants (cv. Longdari 35) treated with azoxystrobin, *Streptomyces* sp. A1022 solid concentrate (SC), and untreated control

Harvest date	Treatment	Fruit number	Fruit fresh wt. (g)	Wt. (g) per pepper fruit
2010. 07. 13.	Azoxystrobin	29	277.5	9.6±0.52
	Streptomyces SC	28	270.0	9.6±0.51
	Untreated	23	221.5	9.5±0.59
2010. 07. 27.	Azoxystrobin	40	473.0	11.8±0.45
	Streptomyces SC	40	499.0	12.5±0.47
	Untreated	61	721.2	11.8±0.60
2010. 08. 24.	Azoxystrobin	208	2525.0	12.1±0.42
	Streptomyces SC	278	3417.5	12.3±0.43
	Untreated	299	3806.7	12.7±0.57
2010. 09. 14.	Azoxystrobin	122	1419.7	11.6±0.47
	Streptomyces SC	130	1521.2	11.7±0.45
	Untreated	109	1213.5	11.2±0.55
2010. 09. 28.	Azoxystrobin	88	884.0	10.0±0.47
	Streptomyces SC	85	945.9	11.1±0.46
	untreated	95	924.6	9.7±0.59
2010. 10. 12.	azoxystrobin	304	2569.5	8.5±0.51
	Streptomyces SC	321	3690.0	11.5±0.49
	untreated	415	3515.7	8.5±0.61

under better growth condition with *Streptomyces* sp. A1022 SC than with azoxystrobin and the untreated control. To further support these findings, the root length of the pepper plant were measured after harvest and found that the average root length was in the following order: *Streptomyces* sp. A1022 SC (28.6±2.3 cm) > azoxystrobin (27.4±3.1 cm) > the untreated control (25.2± 1.3 cm). In addition, the population densities of soil microorganisms (i.e., fungi, actinomycete, and bacteria) at the beginning and end of experiment were also determined. Results showed that the microbial population density in soil with *Streptomyces* sp. A1022 SC remained the same or increased slightly, whereas the density with azoxystrobin and the untreated control decreased by a factor of ten or one hundred (data not shown).

Biocontrol efficacy is determined by the method of application, the number of treatments, and the ability of the selected strains to adapt to different environments. Based on the combined results presented above, *Streptomyces* sp. A1022 and its commercial products could be used for the integrated control of anthracnose diseases of pepper. It is also worth noting that although *Streptomyces* sp. A1022 SC was sprayed in this experiment at a dilution factor of 100 (i.e., 10^7 cfu/mL), the dilution factor could be increased to 1,000 (i.e., 10^6 cfu/mL) if the pepper plants were not severely infected.

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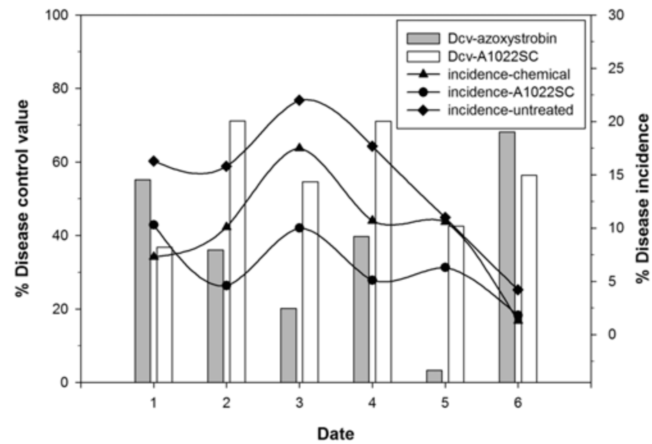

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