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Evaluating the biocontrol efficacy of *Streptomyces plicatus* on Phytophthora crown rot of pepper caused by *Phytophthora capsici*

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ABSTRACT

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Phytophthora capsici is a significant threat to vegetable production in Taiwan and globally. *Streptomyces plicatus* strain B4-7 has emerged as a promising biocontrol agent against Phytophthora blight. Analysis using C18 solid-phase extraction (SPE) fractions of B4-7 in anti-fungal assays against *P. capsici* demonstrated that lower polarity SPE fractions exhibited robust inhibition of mycelial growth and induced abnormal sporangium formation. To use the biological agent B4-7 together with fungicides in crop disease control, we tested the inhibitory effect of five recommended fungicides on B4-7's mycelium growth through paper disc assays. The results showed that, except for chlorothalonil and copper hydroxide, dimethomorph, etriazole, and metalaxyl, exhibited no inhibitory effects on B4-7. When the B4-7 culture broth was applied to Phytophthora-infested peppers, it resulted in 100% inhibition. Similarly, applying copper hydroxide or etriazole alone to the infested plants yielded the same results. Co-application of half-dosages of B4-7 culture broth and copper hydroxide, or etridiazole, significantly reduced the disease severities (12.5%, 0%) and infection rates (12.5%, 0%) of Phytophthora crown rot disease compared to the control treatments (93.8% and 100%) in peppers. These results suggest the potential of combining *S. plicatus* B4-7 with fungicides in an integrated approach for Phytophthora blight management.

Keywords: biocontrol; pesticides; *Streptomyces plicatus*; *Phytophthora capsici*

INTRODUCTION

Phytophthora capsici, a soil-borne pathogen, is a significant threat to vegetable, ornamental, and tropical crops globally [17, 22]. Zoospores of *P. capsici* formed appressorium rapidly within a few hours of reaching the plant host and completed their life cycle in 2 to 3 days under favorable conditions [7, 9]. The oospore, thick-walled sexual spore, can persist in fields without a host, facilitating rapid adaptation of the population to new environments [4, 14].

Disease control strategies encompass breeding resistant host cultivars, employing pathogen-free seedlings, rotating non-host crops, and maintaining well-controlled environments. A crucial aspect is avoiding the overuse of single chemicals and rotating fungicides with different modes of action to mitigate the risk of selecting resistant strains [22]. Several fungicides, including azoxystrobin, copper, dimethomorph, etridiazole, metalaxyl, propamocarb hydrochloride, and zoxamide, have been identified as effective against Phytophthora blight. However, concerns regarding easy accessibility to fungicide application, the development of fungicide resistance, and environmental pollution persist [9]. In response to these concerns, there is a growing interest in biological control agents (BCAs) and non-chemical controls to manage plant diseases and pests, aiming to

reduce overall pesticide usage.

Numerous strains of *Streptomyces* have been identified for their ability to control various plant pathogens, primarily attributed to their antibiotic-producing capabilities [5, 11, 12, 25]. *Streptomyces*, contributing to 80% of the world's antibiotics, encompasses a wide range of properties, including anti-bacterial, anti-fungal, anti-viral, anti-tumoral, anti-hypertensive, and immunosuppressant characteristics [19]. While most known antibiotics are utilized in medical applications for humans and animals, only few of them were applied on plant protection. Direct application of biological control agents (BCAs) into the soil emerges as a more effective management strategy. BCAs may possess additional properties such as mycoparasitism, antibiotic production, chitinase production, and the ability to enrich the rhizosphere. Commercial powder products like *S. lydicus* strain WYEC 108 (Actinovate®) and *S. griseoviridis* strain K61 (Mycostop®) exemplify this approach, demonstrating notable biocontrol efficacy with their dried spores and mycelium [13, 23].

Streptomyces plicatus strain B4-7 has previously demonstrated its anti-*Phytophthora* capabilities in a study by Chen et al. [3]. For the successful integration of B4-7 into an Integrated Pest Management (IPM) program, it is imperative to assess its tolerance to field pesticides. This study aims to evaluate the compatibility between B4-7 and fungicides within cropping systems. Five pesticides were subjected to plate assays to discern their impact on B4-7, with further confirmation conducted through greenhouse trials. The findings provide valuable insights into the adaptability of *S. plicatus* B4-7 within the intricate agro-system, facilitating its effective incorporation into pest management strategies.

MATERIALS AND METHODS

Microorganisms

Streptomyces plicatus strain B4-7 was obtained from the bamboo leaf in a citrus farm located in Qionglin Township, Hsinchu County, Taiwan. The strain maintained on the 2% potato-sucrose-agar medium (PSA: 200 ml decocted potato, 20 g sucrose and 17 g agar per liter of water, pH 7) at 28 ℃. Ten ml distilled water was added into the 7-day-old B4-7 culture plate to wash off the spores, and the spore suspension concentration was adjusted to 1×10^8 cfu/ml for the experiment. A 1 L Erlenmeyer flask (baffled) containing 500 ml of oatmeal broth (OM), prepared with 1% oatmeal (Quaker®, Taiwan) sieved through a 60-mesh sieve, was inoculated with 1 ml of the B4-7 spore suspension. The cultures were incubated at 30 ºC on a shaker (140 rpm) for 6 days. On the 6th day, culture broths were collected for subsequent extraction processes.

Phytophthora capsici isolate 28089 was provided by the Phytophthora Biology Lab (Dept. of Plant Pathology, National Chung Hsing University, Taiwan). *P. capsici* was maintained on V8 juice agar (10% V8 juice, 0.02% $CaCO_3$ and 1.5% agar) at 24 ℃, and grown on fresh media beginning 6 days before the experiment. To induce sporangial formation in *P. capsici*, three agar discs (10 mm in diameter) were transferred into a Petri dish containing 10 ml of sterile distilled water. The dish was exposed to light at a temperature range of 26-28 ℃ for 24 hours. Subsequently, the Petri dish was placed at 4 ℃ for 30 minutes to facilitate the release of zoospores from the sporangia [8].

Anti-*Phytophthora* efficacy of the B4-7 SPE fractions

The procedure involved the concentration and purification of the anti-fungal compound from four liters of B4-7 OM culture filtrate. The culture filtrate was concentrated to 40 ml using a rotary evaporator (Laborota 4000 efficient, Heidolph instruments, Germany) at 45 ℃. The concentrated solution was divided into four portions, each adjusted to different pH values (5, 6, 7, and 8) with phosphoric acid before undergoing the purification process. Solid-phase extraction columns [Bakerbond™ C18 SPE Octadecyl disposable extraction columns 500 mg/ 6 ml, J.T.Baker, USA] prepared by washing with methanol (Baker analyzed® HPLC solvent, J.T.Baker, USA) three times, followed by distilled water adjusted to the same pH value. A 10 ml sample was loaded into the SPE column, and gradient of water/methanol solutions was added to create decreasing polarity. Each pH treatment yielded 11 fractions, and each fraction was concentrated using a condenser. Each pH treatment had 11 fractions and each fraction was concentrated by the condenser. The SPE fractions were re-dissolved in 0.5 ml 50% methanol for plate assay to qualitatively assess the anti-microbial efficacy.

The anti-fungal test involved creating two 4 mm diameter holes on the edge of a V8 juice agar plate, adding 20 µl of the SPE fraction to each hole, and placing a 10 mm *P. capsici* isolate 28089 agar disc in the middle of the plate. A 20 µl 50% methanol was used as the control. Plates were incubated at 24 ℃, and daily observations were made until the mycelia of the control treatment reach the edge of hole. The clear area between the edge and the mycelia was measured to determine the anti-fungal efficacy of the SPE fraction. The inhibition rate (%) = (A-B)/A × 100; A means the length between the hole and fungal disc, B means the fungal colony radius. Each treatment had two replicates, and the experiment was repeated two times using SPE fractions from two different culture broth batches.

Effects of pesticides on B4-7 on plate assays

Five fungicides commonly used for Phytophthora blight were tested, including chlorothalonil (75% wettable powder, Lanlix cropscience), copper hydroxide (77% WP, Chiapin trade), etridiazole (25% emulsifiable concentrates, Uniroyal Chemical Taiwan), dimethomorph (50% WP, Jia Non Enterprise), and metalaxyl (35% WP, Fulon chemical). The pesticides were prepared in three dosages according to the recommended label rates [18]: the recommended concentration, double concentration, and half of the recommended concentration. The double and half concentrations were utilized to replicate scenarios of pesticide accumulation and decline in the field.

The toxicity of pesticides to B4-7 was evaluated using filter paper discs through two distinct methods, mimicking scenarios where the BCA was applied simultaneously with pesticides or when the BCA population was already established. In the first method, B4-7 spore suspensions (200 µl) were uniformly spread on PSA plates using an inoculation loop. Subsequently, three paper discs (8 mm in diameter, thick, Advantec®, Tokyo, Japan), each containing 50 µl of a pesticide or distilled water (mock control), were placed on the top of the plate. The plates were then incubated at 28 ℃ for 7 days. In the second method, B4-7 was cultured on PSA at 28 ℃ for 24 h, and paper discs containing 50 µl of a pesticide or distilled water were placed on the top of B4-7 lawn. After 6 days of incubation, the plates were examined for inhibitory zones. All experiments were repeated three times, and each treatment included at least three replicates.

The BCA reactions around the paper discs were categorized into five levels. Level 0: BCA grew well and produced spores normally, indicating no inhibitory effects. Level 1: BCA grew normally but produced fewer spores; no apparent inhibition zones around the paper disc. Level 2: BCA grew less abundantly, showing inhibitory halos around the paper disc. Level 3: BCA grew sporadically, with or without blurred zones. Level 4: BCA failed to grow, displaying a distinct inhibitory zone around the paper disc.

Effects of B4-7 and pesticides on controlling *P. capsici* in greenhouse trials

Bell pepper seeds (*Capsicum annuun* L. cv. California Wonder; R. H. Shumway, USA) were initiated for germination by placing them in a glass Petri dish alongside a wet paper towel. The 10-day-old seedlings were transplanted into 60 ml pots filled with the soil mixture (peat: perlite = 3:1) containing a soil mixture of peat and perlite in a ratio of 3:1. The plants were cultivated in a greenhouse under natural sunlight, maintaining a temperature range of 26-28 ℃. To support plant growth, the pepper plants received weekly fertilization with Hyponex No. 2 (with a ratio of K: P: N = 20: 20: 20; Hyponex Corporation, Ohio, USA). After five weeks of growth, the pepper plants were deemed ready for the experiment.

Two pesticides, copper hydroxide (1925 ppm) and etridiazole (100 ppm) were tested and the concentration and application methods of pesticides were selected based on the recommendations in the Plant Protection Manual [18]. A zoospore suspension of *P. capsici* isolate 28089 (1×10^5 zoospores) in 200 µl was evenly inoculated into three holes (0.4 cm diameter × 1.0 cm depth) around the bell pepper, three hours prior to other treatment. Treatments were organized into six groups. Group 1: the infested pots were drenched with 20 ml tap water, serving as the control treatment. Group 2: the infested pots were drenched with 20 ml B4-7 OM culture broth (B4-7). Group 3: the infested pots were drenched with 20 ml pesticide (P). Group 4: the infested pots were drenched with 20 ml pesticide 24 h after inoculation (P-24h). Group 5: the infested pots were drenched with 20 ml of equal volume mixture of B4-7 culture broth and pesticide 24 h after inoculation (B4-7 + P). Group 6: the infested pots were drenched with 20 ml of equal volume mixture of B4-7 culture broth and tap water 3 h after inoculation, and then with 20 ml of equal volume mixture of pesticide and tap water 24 h after inoculation (B4-7 + P-24h). Disease severity was assessed six days after pathogen inoculation using crown rot symptom scale ranging from 0-4 [21]. Zero means health plant; 1 = crown rot symptom less than 10 mm on the stem base (Fig. 3A); 2 = 11-20 mm; 3 = 21-30 mm; and 4 means the rot symptom more than 30 mm from the basal stem. The disease severity (%) was calculated as follow: [Σ (scale × plant numbers) / (highest scale × total plant numbers)] × 100 [10]. The infection rate (%) was calculated as follow: disease plant numbers/ total plant numbers × 100. Each treatment had 4 replicates and randomized placed on the greenhouse bench under natural sunlight, maintaining a temperature range of 26-28 ℃. The experiment was repeated twice.

Statistical analysis

The data from two greenhouse trial repeats were combined, and the effects of each pesticide treatment were analyzed separately. An analysis of variance (ANOVA) was performed according to Tukey's b test ($P = 0.05$) available in IBM SPSS Statistics version 20.0 (New York, USA). Data of disease severity and infection rate were arcsine transformed before statistical analyses.

RESULTS

The anti-fungal efficacy of B4-7 SPE fractions

Each pH value yielded eleven fractions, totaling 44 fractions collected from C18 SPE. These fractions were dissolved in 0.5 ml

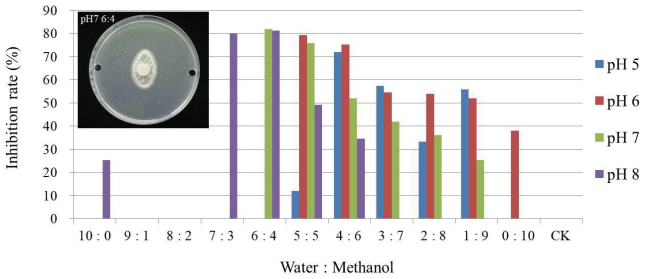

Fig. 1. The Anti-*Phytophthora capsici* activities of C18 SPE fractions separated from *S. plicatus* B4-7 oatmeal culture broth at four pH value backgrounds.

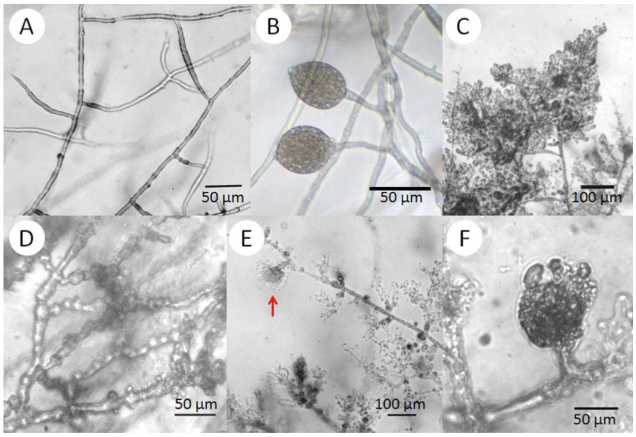

Fig. 2. Effects of the *S. plicatus* B4-7 fractions on *P. capsici* isolate 28089 mycelia and sporangia. (A) Normal mycelia. (B) Normal sporangia. (C, D) Abnormal mycelia and branching. (E) Abnormal mycelia with apical hyphae lysis (arrow). (F) Abnormal sporangium.

of 70% MeOH for the in vitro test. The test results revealed that antifungal compounds were present in the fractions eluted from the portion with higher MeOH (Fig. 1). The treatments with pH 7 and pH 8 fraction 6:4 (water: MeOH) filtrates exhibited the highest *P. capsici* isolate 28089 mycelial inhibition rates at 82.5% and 81.8%, respectively. Abnormal *P. capsici* isolate 28089 mycelia and sporangia were observed under a microscope (Fig. 2). Among the four pH values, fractions from the pH 6 and pH 7 conditions showed better separation compared to the pH 5 and pH 8 conditions because fractions from pH 6 and pH 7 exhibited superior anti-fungal activities.

Effects of fungicides on B4-7 on plate assays

Among the five fungicides tested, both dimethomorph and etridiazole showed no inhibitory to the growth of B4-7, regardless of the application sequences. Chlorothalonil and copper hydroxide exhibited a level 4 inhibitory effect on B4-7, with inhibition zones measuring 2.0 and 3.3 cm in diameter, respectively (Table 1). Metalaxyl produced a level 2 inhibition zone with a diameter of 1.2 cm when applied simultaneously with B4-7. These fungicides demonstrated lower inhibitory effects when B4-7 had been inoculated for 24 hours.

Effects of B4-7 and pesticides on controlling *P. capsici* in greenhouse trials

When the bell pepper inoculated with *P. capsici* isolate 28089 was treated with B4-7 OM culture broth, the infection rate was 0%, in contrast to the water control, which exhibited a 100% infection rate (Table 2, Fig. 3). Copper hydroxide and etridiazole (P) both demonstrated a 0% infection rate in the experiment. When B4-7 was applied with copper hydroxide, regardless of a 24-hour interval or not, it showed a 12.5% disease severity and a 12.5% infection rate. Similarly, when B4-7 was applied with etridiazole, all results showed a 0% infection rate (Table 2).

Discussion

TABLE 1. Fungicides and their effects on the growth of *Streptomyces plicatus* B4-7 assayed on plates using paper discs.

Common name	Mode of action	Category	Concentration (µg/ml)[a]	Inhibition Level[b]	Inhibition zone (cm)[c]
Chlorothalonil	Multi-site contact activity	Chloronitriles	3750	4 (1)	2.0
Copper hydroxide*[d]		Inorganic copper	3850	4 (0)	3.3
Dimethomorph	Phospholipid biosynthesis and cell wall deposition	Carboxylic acid amide	500	0 (0)	
Etridiazole	Lipid peroxidation	Triazoles	250	0 (0)	
Metalaxyl	RNA polymerase I	Phenylamide	350	2 (0)	1.2

[a] The concentration of active ingredient in the pesticide. Three concentrations of each pesticide were tested, this table only shown the results of the highest concentration in the experiment.

[b] Inhibition levels (0-4) as mention in M&M. Number in parentheses is an inhibition level of a pesticide applied 24 h after B4-7 was grown.

[c] Means of nine replicates. Inhibition zone diameter was including the paper disc (0.8 cm).

[d] * The inhibition level did not change when the pesticide concentration was reduced.

TABLE 2. Evaluation of the effects of *S. plicatus* B4-7 applied with 2 fungicides in two different orders for controlling *P. capsici* isolate 28089 in bell pepper.

Treatment[a]	Disease severity (%)[b]		Infection rate (%)[c]	
Control-water	93.8 a[d]	A	100.0 a	A
B4-7	0.0 b	B	0.0 b	B
Copper hydroxide				
P	0.0 b		0.0 b	
B4-7 + P	12.5 b		12.5 b	
P-24h	0.0 b		0.0 b	
B4-7 + P-24h	12.5 b		12.5 b	
Etridiazole				
P	0.0	B	0.0	B
B4-7 + P	0.0	B	0.0	B
P-24h	12.5	B	12.5	B
B4-7 + P-24h	0.0	B	0.0	B

[a] P: pesticide; B4-7: *S. plicatus* B4-7 culture broth.

[b] Disease severity (%) = [Σ (scale × plant numbers) / (highest scale × total plant numbers)] ×100.

[c] Infection rate (%) = diseased plant numbers / total plant numbers ×100.

[d] The data presented is an average of two trials. Means (n = 8) in the same column followed by the same letter are not significantly different (P = 0.05) according to Tukey's b test. Data were arcsine transformed before statistical analyses.

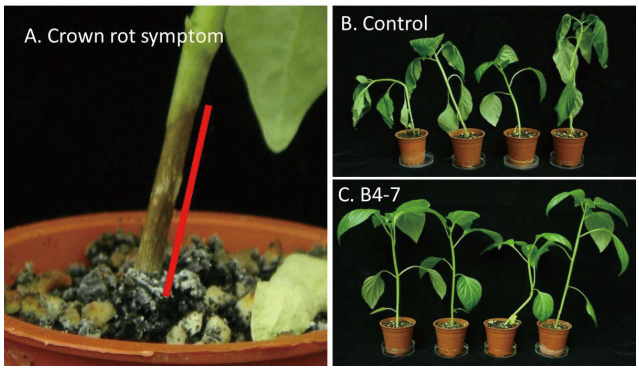

Fig. 3. Effects of the *S. plicatus* B4-7 oatmeal culture broth on *P. capsici* isolate 28089 on bell pepper. (A) Crown rot symptom. (B) Control treatment. (C) *S. plicatus* B4-7 oatmeal culture broth treatment.

Streptomyces plicatus B4-7 demonstrated inhibition of the mycelium growth of *P. capsici*, *P. cinnamomi*, *P. palmivora* and *P. parasitica* in the dual culture plate assays, along with observed hyperparasitism on *P. capsici* [3]. To purify secondary metabolites of B4-7 from the oatmeal culture broth, a reverse-phase (C18 SPE) tactic was employed, and the lower polarity extracts from SPE exhibited anti-*Phytophthora* activities. The antibiotic compound identified as borrelidin in a previous study demonstrated inhibitory effects on mycelium growth and zoospore germination [3]. Originally isolated from *S. rochei* [2], borrelidin has been studied for its antibacterial, antifungal, anticancer, antimalarial, herbicidal, and insecticidal properties [2, 6]. In previous studies, *S. plicatus* was found to produce chitinase, endo-beta-N-acetylglucosaminidase H (Endo H), and several antibiotics [1, 3, 15, 16, 24].

This study examined the compatibility between *S. plicatus* B4-7 and various fungicides. Plate assays indicated that the tested fungicides had no significant impact on the growth of B4-7 at recommended dosages. Notably, copper hydroxide exhibited a Level 4 inhibitory effect at different dosages, but when applied 24 hours after B4-7 growth on the plate, no inhibition was observed. In greenhouse trials, the combined application of half the recommended dosage of copper hydroxide or etridiazole with B4-7 culture broth resulted in a significant reduction in Phytophthora crown rot disease severities and infection rates compared to the control treatment. These findings suggest that B4-7 can be effectively used in combination with pesticides, expanding its potential applications in Phytophthora blight management.

Microorganisms with biocontrol abilities can be highly beneficial for crop management programs. The application of these microorganisms has the potential to enrich soil nutrients, enhance plant growth, control plant pathogens, and reduce the need for pesticides. In practical terms, the use of *S. saraceticus* strain 31 in combination with LTM (a commercial product comprising soybean meal, crab and shrimp shells, neem seed oilcake, sugar, and dolomite as a soil amendment) has shown positive effects. This combination has been found to enhance pH levels, increase inorganic phosphate and organic matter contents, and reduce soil electrical conductivity (EC) values [20]. Moreover, in pot and greenhouse trials, the combined application of SS31 and LTM demonstrated a significant reduction in both *Rhizoctonia solani* and *Meloidogyne incognita* [20].

In conclusion, this study highlights the versatility of *S. plicatus* B4-7, demonstrating its effective use in combination with various fungicides or coexistence with fungicide residues. Fungicides, when employed during pathogen outbreaks, can reduce disease incidence, while beneficial microorganisms, once stably established in the rhizosphere, provide long-term pest control and support healthy root growth. The findings from this research offer valuable insights for broadening the application and optimizing the efficacy of *S. plicatus* B4-7 in managing Phytophthora blight. The integration of beneficial microorganisms into Integrated Pest Management (IPM) programs has the potential to enhance food safety by diminishing reliance on potentially harmful pesticides, contributing to more sustainable agricultural practices.

REFERENCES

1. Baharlouei, A., Sharifi-Sirchi, G. R., and Shahidi Bonjar, G. H. 2010. Identification of an antifungal chitinase from a potential biocontrol agent, ***Streptomyces plicatus*** strain 101, and its new antagonistic spectrum of activity. Philipp. Agric. Sci. 93: 439-445.
2. Berger, J., Jampolsky, L. M., and Goldberg, M. W. 1949. Borrelidin, a new antibiotic with anti-borrelia activity and penicillin enhancement properties. Arch. Biochem. 22: 476-478.
3. Chen, Y. Y., Chen, P. C., and Tsay, T. T. 2016. The biocontrol efficacy and antibiotic activity of ***Streptomyces plicatus*** on the oomycete ***Phytophthora capsici***. Biol. Control 98: 34-42.
4. Erwin, D. C., and Ribeiro, O. K. 1996. Phytophthora diseases worldwide. American Phytopathological Society, St. Paul, MN.
5. Faheem, M., Raza, W., Zhong, W., Nan, Z., Shen, Q., and Xu, Y. 2015. Evaluation of the biocontrol potential of ***Streptomyces goshikiensis*** YCXU against ***Fusarium oxysporum*** f. sp. ***niveum***. Biol. Control 81: 101-110.
6. Fang, P., Yu, X., Jeong, S. J., Mirando, A., Chen, K., Chen, X., Kim, S., Francklyn, C. S., and Guo, M. 2015. Structural basis for full-spectrum inhibition of translational functions on a tRNA synthetase. Nat. Commun. 6: 6402.
7. Judelson, H. S. and Blanco, F. A. 2005. The spores of ***Phytophthora***: weapons of the plant destroyer. Nat. Rev. Microbiol. 3: 47-58.
8. Ko, W. H., Tsou, Y. J., Lin, M. J., and Chern, L. L. 2010. Activity and characterization of secondary metabolites produced by a new microorganism for control of plant diseases. N. Biotechnol. 27: 397-402.
9. Lamour, K. H., Stam, R., Jupe, J., and Huitema, E. 2012. The oomycete broad-host-range pathogen ***Phytophthora capsici***. Mol. Plant Pathol. 13: 329-337.
10. Li, C. H., Shi, L., Han, Q., Hu, H. L., Zhao, M. W., Tang, C. M., and Li, S. P. 2012. Biocontrol of verticillium wilt and colonization of cotton plants by an endophytic bacterial isolate. J. Appl. Microbiol. 113: 641-651.
11. Li, Q., Jiang, Y., Ning, P., Zheng, L., Huang, J., Li, G., Jiang, D., and Hsiang, T. 2011. Suppression of ***Magnaporthe oryzae*** by culture filtrates of ***Streptomyces globisporus*** JK-1. Biol. Control 58: 139-148.
12. Li, Q., Ning, P., Zheng, L., Huang, J., Li, G., and Hsiang, T. 2012. Effects of volatile substances of ***Streptomyces globisporus*** JK-1 on control of ***Botrytis cinerea*** on tomato fruit. Biol. Control 61: 113-120.
13. Minuto, A., Spadaro, D., Garibaldi, A., and Gullino, M. L. 2006. Control of soilborne pathogens of tomato using a commercial formulation of ***Streptomyces griseoviridis*** and solarization. Crop Prot. 25: 468-475.
14. Quesada-Ocampo, L. M., Parada-Rojas, C. H., Hansen, Z., Vogel, G., Smart, C., Hausbeck, M. K., Carmo, R. M., Huitema, E., Naegele, R. P., Kousik, C. S., Tandy, P., and Lamour, K. 2023. ***Phytophthora capsici***: recent progress on fundamental biology and disease management 100 years after its description. Annu. Rev. Phytopathol. 61: 185-208.
15. Robbins, P. W., Trimble, R. B., Wirth, D. F., Hering, C., Maley, F., Maley, G. F., Das, R., Gibson, B. W., Royal, N., and Biemann, K. 1984. Primary structure of the ***Streptomyces*** enzyme endo-beta-N-acetylglucosaminidase H. J. Biol. Chem. 259: 7577-7583.
16. Robbins, P. W., Albright, C., and Benfield, B. 1988. Cloning and expression of a ***Streptomyces plicatus*** chitinase (chitinase-63) in ***Escherichia coli***. J. Biol. Chem. 263: 443-447.
17. Wang, L. and Ji, P. 2021. Fitness and competitive ability of field isolates of ***Phytophthora capsici*** resistant or sensitive to fluopicolide. Plant Dis. 105: 873-878.
18. Wang, Y. C., Wang, T. C., Chen, F. S., Tsai, Y. S., Li, H. P., and Fei, W. C. 2012. Plant Protection Manual. Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture, Taichung, Taiwan. 1079 pp.
19. Watve, M. G., Tickoo, R., Jog, M. M., and Bhole, B. D. 2001. How many antibiotics are produced by the genus ***Streptomyces***? Arch. Microbiol. 176: 386-90.
20. Wu, P. H., Tsay, T. T., and Chen, P. 2021. Evaluation of ***Streptomyces saraciticas*** as soil amendments for controlling soil-borne plant pathogens. The Plant Pathol. J. 37(6): 596-606.
21. Segarra, G., Aviles, M., Casanova, E., Borrero, A., and Trillas, I. 2013. Effectiveness of biological control of ***Phytophthora capsici*** in pepper by ***Trichoderma asperellum*** strain T34. Phytopathol. Mediterr. 52, 77-83.
22. Siegenthaler, T. B. and Hansen, Z. R. 2021. Sensitivity of ***Phytophthora capsici*** from Tennessee to mefenoxam, fluopicolide, oxathiapiprolin, dimethomorph, mandipropamid, and cyazofamid. Plant Dis. 105: 3000-3007.
23. Tokala, R. K., Strap, J. L., Jung, C. M., Crawford, D. L., Salove, M. H., Deobald, L. A., Bailey, J. F., and Morra, M. J. 2002. Novel plant-microbe rhizosphere interaction involving ***Streptomyces lydicus*** WYEC108 and the pea plant (***Pisum sativum***). Appl. Environ. Microbiol. 68: 2161-2171.
24. Trumbly, R. J., Robbins, P. W., Belfort, M., Ziegler, F. D.,

Maley, F., and Trimble, R. B. 1985. Amplified expression of *Streptomyces* endo-beta-N-acetylglucosaminidase H in *Escherichia coli* and characterization of the enzyme product. J. Biol. Chem. 260: 5683-5690.

25. Ziedan, E. S. H., Farrag, E. S., El-Mohamedy, R. S., and Abd-Alla, M. A. 2010. *Streptomyces alni* as a biocontrol agent to root-rot of grapevine and increasing their efficiency by biofertilisers inocula. Arch. Phytopathol. Plant Prot. 43: 634-646.

摘　要

陳盈宇*、蔡東纂、陳珮臻。2024。鏈黴菌*Streptomyces plicatus*對*Phytophthora capsici*引起青椒冠腐病之生物防治效用探討。植物醫學66(1_2): 7-14。

疫病菌*Phytophthora capsici*對台灣和全球的蔬果生產都構成重大威脅。放線菌*Streptomyces plicatus* strain B4-7為具有抑制疫病菌的生物防治微生物，將B4-7燕麥培養液經過C18固相萃取管分離不同極性的濾液，並與*P. capsici* isolate 28089進行拮抗試驗。拮抗試驗數據顯示，極性較低的SPE濾液對疫病菌絲生長具有強烈的抑制作用，並造成疫病菌絲與孢囊的型態變異。為了讓B4-7能運用到作物病害綜合防治策略中，我們透過圓盤濾紙試驗，評估五種疫病常用的殺菌劑對B4-7菌落生長影響。結果顯示，除了四氯異苯腈和氫氧化銅之外，達滅芬、依得利和滅達樂皆對B4-7的生長不具有抑制作用。進一步將B4-7燕麥培養液與殺菌劑分別、或混合施用於盆缽試驗，觀察對青椒冠腐病的防治效果。實驗數據顯示，單獨施用B4-7發酵液時，對青椒冠腐病的抑制率為100%。在單獨施用氫氧化銅，或依得利的處理組亦是100%的抑制率。在B4-7+依得利混合處理組(皆濃度減半)的疾病嚴重度為0%，感染率0%，與對照組(93.8%、100%) 有顯著抑制病害發展。在B4-7+氫氧化銅(皆濃度減半) 處理組的疾病嚴重度則為12.5%，感染率12.5%，同樣具有顯著抑制效果。綜合上述結果，本研究所評估之*S. plicatus* B4-7具有非農藥防治青椒疫病、降低作物損失、降低用藥劑量的潛力，可作為青椒疫病管理策略的選項之一。

關鍵詞：生物防治、農藥、鏈黴菌、疫病菌