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Research Article

Biocontrol of *Phytophthora infestans*, Fungal Pathogen of Seedling Damping Off Disease in Economic Plant Nursery

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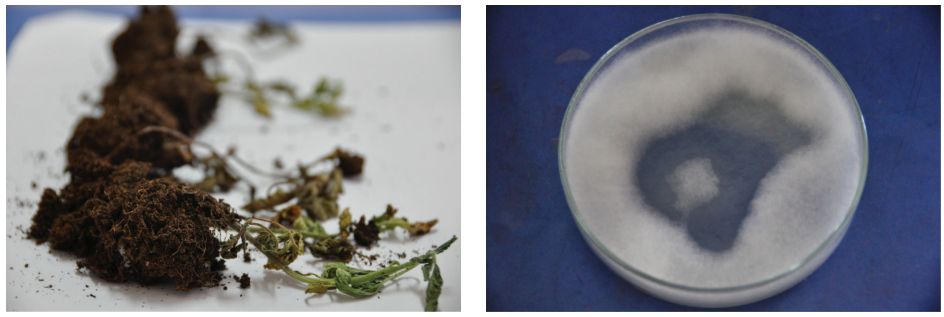
This research aims to control Seedling damping off disease in plants by using antagonistic actinomycetes against the causative fungi. *Phytophthora infestans* was isolated from the infected tomato plant seedling obtained from an economic plant nursery in Amphoe Pak Chong, Nakhon Ratchasima Province, Thailand. The chitinolytic *Streptomyces rubrolavendulae* S4, isolated from termite mounds at the grove of Amphoe Si-Sawat, Kanchanaburi Province, Thailand, was proven to be the most effective growth inhibition of fungal pathogens tested on potato dextrose agar. Tomato and chili seedlings that colonized with antagonistic *S. rubrolavendulae* S4 were grown in *P. infestans* artificial inoculated peat moss. Percents of noninfested seedling in fungal contaminated peat moss were compared to the controls with uninoculated peat moss. In *P. infestans* contaminated peat moss, the percents of survival of tomato and chili seedling were significantly increased ($P < 0.05$) from 51.42 to 88.57 and 34.10 to 76.71 for the *S. rubrolavendulae* S4 treatment, respectively. The *S. rubrolavendulae* S4 also showed high efficiency equivalent to fungicide, metalaxyl with no significant difference ($P > 0.05$). It was clearly demonstrated that *S. rubrolavendulae* S4 can prevent the tomato and chili seedling damping off disease in economic plant nurseries.

1. Introduction

The value of vegetable crops in Thailand was estimated to be around 14,561 million baht in 2009, including tomato and chili. The plantation of these economic crops is done by using reliable seedling producers. Therefore, the economic plant nursery business has been increasing. Disease management has become a major concern during the production of vegetable plug transplants. The seedling damping off disease causes serious problems in economic plant nurseries. Causative pathogenic fungi of seedling damping off disease in plants were reported to be *Pythium* spp., *Phytophthora* sp. [1, 2], *Rhizoctonia solani* [3], *Sclerotium rolfsii* [4], and *Fusarium oxysporum* [5]. *Phytophthora infestans* is the most infamous species of genus which caused pre- and postemergence damping-off and late blight of potato and tomato. Also, peppers, melons, pumpkins, citruses, strawberries, chestnuts, and forest trees are affected by *Phytophthora* species such as *P. cambivora, P. hibernalis, P. citrophthora, P. kernoviae, P. capsici, P. cactorum, P. drechsleri,* and *P. infestans* [6–9]. Chemical fungicides are extensively used in current agriculture and also cause environmental pollution. Nowadays, a method of controlling or preventing the disease is by decreasing hazardous chemical fungicides. Biocontrol is used as an alternative method. The microorganism simultaneously grows together with pathogenic fungi and produced enzyme or organic compounds for suppression fungal growth. Biocontrol with microbial fungicides is being investigated in several academic labs. Seedling damping off disease occurred in economic plant nurseries in Amphoe Pak Chong, Nakhon Ratchasima Province. In this study, the major causative fungal pathogens of the seedling damping disease were investigated. The antagonistic chitinolytic *Streptomyces* against the fungal pathogen was experimented to be used to control the disease in plant nurseries.

2. Materials and Methods

2.1. Isolation and Identification of Plant Pathogenic Fungi. The plant samples were obtained from economic plant



(a) (b)

Figure 1: *Phytophthora infestans* isolated from infected tomato plant seedling (a) and produced white, profusely branching mycelium (b).

nurseries. Roots and stems of tomato seedlings with damping off disease symptoms were washed to remove any excess peat moss. Then, the infested plant parts were surface-sterilized using 5% v/v hypochlorite for 30 seconds, washed with sterile water, and blot-dried on sterile filter paper. Plant pieces were cut into 0.5 cm lengths before being placed onto potato dextrose agar (PDA). The fungal mycelium and spores that grew out of the plant tissues were subcultured and purified on another PDA plate. The pathogenic fungi were identified based on colony morphology and by the characteristics of sporangium and oospores.

2.2. Antagonistic Actinomycetes. The chitinolytic actinomycete was isolated from the termite mounds at the grove of Amphoe Si-Sawat, Kanchanaburi Province, Thailand, by using the soil dilution method. The chitinolytic actinomycete was preliminary classified to be *Streptomyces* sp. based on morphological and physiological characteristics [10]. The chitinolytic actinomycete was found similar to *Streptomyces rubrolavendulae* based on 16S rRNA analysis. The *S. rubrolavendulae* S4 was maintained on nutrient agar slants at 30°C for 3–5 days to make the fresh colony before being used in the next experiment.

2.3. Antagonistic Test by Dual Culture Technique. The antifungal activity of the *S. rubrolavendulae* S4 against seedling damping off fungi was tested on V8 agar [11] at 30°C using a dual culture technique. The conidia of the *S. rubrolavendulae* S4 were placed on a V8 agar plate in lines. Then, the plates were incubated at 30°C for 5 days to allow growth and sporulation of the *S. rubrolavendulae* S4 prior to inoculation of an agar plug of the pathogenic fungi at the center of the plate. After incubation for 3–5 days at 30°C, the growth inhibition of pathogenic fungi by *S. rubrolavendulae* S4 was investigated. The size of the zone of inhibition developing around the *S. rubrolavendulae* S4 was a measurement of the antagonistic potential against the pathogen. Only the pathogen was used as a positive control and the experiments were repeated three times with three replications for each experiment. Percentage growth inhibition was determined after 3 days incubation by this formula of Skidmore [12]:

$$\text{The percentage of inhibition growth } (\%) = \frac{R - r}{R} \times 100, \quad (1)$$

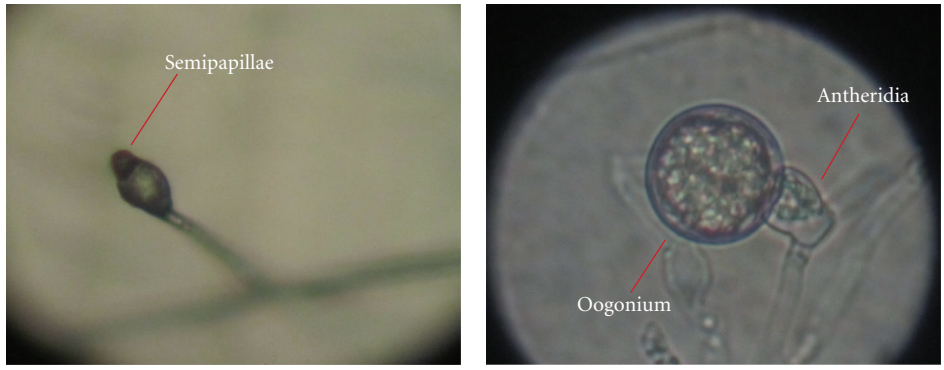
where R represents the radius of the control pathogens growth and r the radius of the pathogen's growth towards the bacterial antagonist.

2.4. Suppression of Seedling Damping Off Fungi under Greenhouse Conditions. Peat mosses were sterilized for 15 mins at 121°C 15 lbs/in^2 three times at 24 h intervals and used as a planting material in this study. The agar plugs, taken from the edge of the young colony of pathogenic fungi, were artificial inoculated into steam-pasteurized peat mosses at the rate of 50 agar plugs/250 g. *S. rubrolavendulae* S4 was cultured in nutrient broth with 1% w/v shrimp shell powder at 30°C for 3 days and used for plant protection experiments. The *S. rubrolavendulae* S4 cell suspension was inoculated into the peat moss at the final concentration of 10^6 cfu/g. The experiment was a 2 × 5 factorial completely randomized design with three replicates. Two kinds of seedling were used: tomato and chili. The 3 sets of 10 seedlings were grown in five types of treated planting material: (1) artificial fungal pathogen infested, (2) artificial fungal pathogen infested but challenged with *S. rubrolavendulae* S4, (3) artificial fungal pathogen infested but treated with fungicide, metalaxyl (Phyto-Q), *S. rubrolavendulae* S4 inoculated, and (4) uninoculated planting material as control. Percentages of the noninfested seedling were then determined.

3. Results

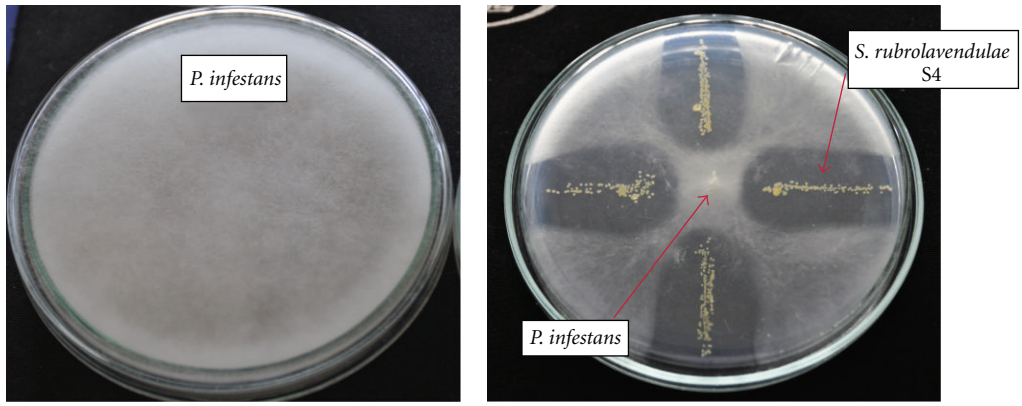
3.1. The Causative Fungal for Damping Off Disease of Seedlings in the Plant Nursery. The major plant pathogenic fungi isolated from the infected tomato plant seedling was identified to be *Phytophthora infestans,* based on morphology in the form of chlamydospores and sporangia which produce zoospores [13]. This isolate can be grown in PDA and produced white, profusely branching and aseptate mycelium without septum (Figure 1).

The leading edge of mycelia plugs was transferred to petri dishes containing peat moss extract, and then incubated



(a) (b)

Figure 2: *Phytophthora infestans*, (a) lemon shape sporangia, (b) amphigynous antheridia of oospores.



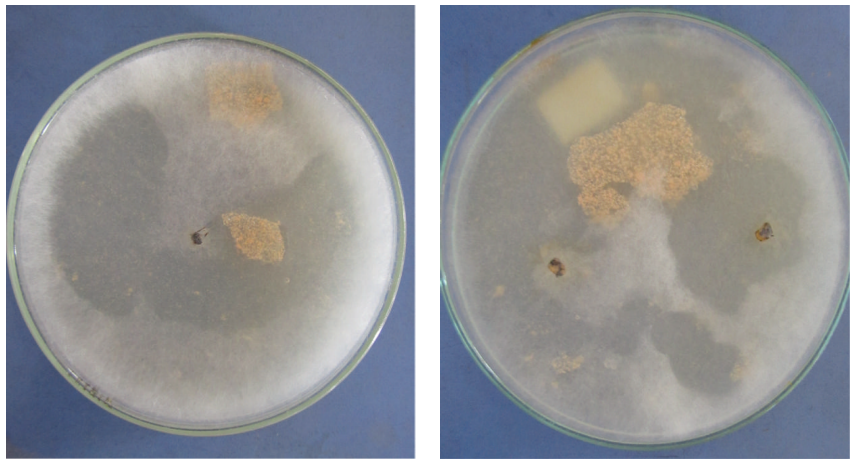
(a) (b)

Figure 3: *In vitro* interactions between *S. rubrolavendulae* S4 and *Phytophthora infestans* on V8 medium. (a) Control plate of *Phytophthora infestans*; (b) *S. rubrolavendulae* S4 against *Phytophthora infestans*.

at room temperature under continuous 40-watt fluorescent illumination for between 1 and 4 days, amphigynous antheridia of oospores. The sporangia in a lemon shape were observed (Figure 2) which are a semipapillae type of sporangia and released zoospores in wet peat moss or water.

3.2. Antagonistic Activity of S. rubrolavendulae S4 against P. infestans. The dual culture method that was used to investigate the antagonistic of the *S. rubrolavendulae* S4 indicated that *S. rubrolavendulae* S4 that was used as antagonistic microorganism for suppression mycelia growth of *P. infestans* on V8 Agar (Figure 3). After being incubated for 3 days at room temperature, the radiuses of *P. infestans* growth on control plate and *P. infestans* growth toward *S. rubrolavendulae* S4 were measured about 4.5 cm and 0.75 cm, respectively. Moreover, the radial growth of *P. infestans* produced a clear zone around the *S. rubrolavendulae* S4 growth indicating the inhibition of the fungal growth. Therefore, 83.33% of growth inhibition has clearly demonstrated that *S. rubrolavendulae* S4 exhibited good growth inhibition of the pathogenic fungi, *P. infestans*.

3.3. Suppression of Tomato and Chili Seedling Damping Off Disease by Antagonistic S. rubrolavendulae S4. The biological suppression of the seedling damping off disease of tomato and chili seedling was performed. *S. rubrolavendulae* S4 cultured in shrimp shell broth at optimum conditions was inoculated into satirized peat moss. Tomato and chili seedling were grown in peat moss and colonized with antagonistic *S. rubrolavendulae* S4 and *P. infestans*. Results from the greenhouse pot experiment demonstrated that *S. rubrolavendulae* S4 significantly inhibited root rot of tomato and chili seedling caused by *P. infestans*. Percents of noninfested seedling in fungal contaminated peat moss were compared to the controls with uninoculated peat moss. In *P. infestans* contaminated peat moss, the percents of survival of tomato and chili seedling were significantly increased ($P < 0.05$) from 51.42 to 88.57 and 34.10 to 76.71 for the isolate S4 treatment, respectively (Table 1). The *S. rubrolavendulae* S4 also showed a high efficiency equivalence to fungicide, metalaxyl with no significant difference ($P > 0.05$). These treated plants looked healthy and increased the percentage of healthy plants showing no symptoms of root rot. The *P. infestans* was reisolated from the infested seedling to confirm the effectiveness of the fungal pathogen.



(a) (b)

Figure 4: *Phytophthora infestans* was observed from peat moss (a) and seeds (b).

Table 1: Efficacy of biocontrol, *S. rubrolavendulae* S4 on suppression of tomato and chili seedling damping off disease caused by *Phytophthora infestans* under greenhouse conditions.

Treatment	Percentage of noninfested tomato seedling	Percentage of noninfested chili seedling
Control	88.56[b]	95.71[c]
P. infestans	51.42[a]	34.10[a]
P. infestans + *S. rubrolavendulae* S4	88.57[b]	76.71[b]
P. infestans + Phyto-Q	94.28[b]	79.99[b]
S. rubrolavendulae S4	87.14[b]	90.29[bc]

[a, b, c] Means within a column with the same letter were not significantly different ($P > 0.05$).

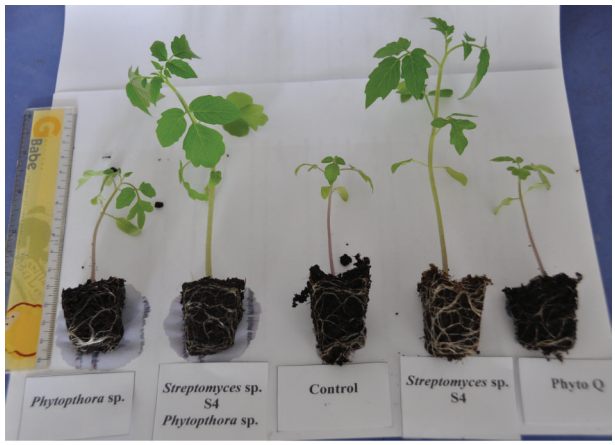


Figure 5: The healthy tomato seedlings grown in peat moss inoculated with *Phytophthora infestans* and *S. rubrolavendulae* S4 in different treatments.

4. Discussion

The plant pathogenic fungi, *Phytophthora infestans,* was isolated from the infected tomato plant seedling in the economic plant nursery. *Phytophthora* often called water mold can be grown in wet soil and produced white, profusely branching, aseptate mycelium, sporangia, and oospores. This *Phytophthora* can spread widely with zoospores and oospores which are produced in sporangium and oogonium, respectively [14].

In sexual spore type, oospores were produced when antheridia was attached to oogonium. Moreover, asexual spore types of *P. infestans* are chlamydospores and sporangia were used as a survival structure. The zoospores were contained in a lemon shape of sporangium (Figure 2(a)).

In the plant fields, greenhouses, and nurseries, chemical fungicides were used for disease management. Metalaxyl and fosetyl-A1 are suggested chemical fungicides to be used against *Phytophthora* species which are dangerous for the environment [15–18]. Therefore, the biological control was applied for disease management that will be safer for health and the environment. Control of *Phytophthora* root rot was achieved by infesting peat moss with *Streptomyces* at the time of planting under greenhouse conditions.

The frequency of healthy plants increased significantly for the susceptible variety, and the average disease severity index decreased significantly for both the resistant and susceptible varieties tested. It was clearly demonstrated that isolate S4 could prevent the tomato and chili seedling damping off disease in the economic plant nursery. In recent studies, the *Trichoderma harzianum*, *Bacillus subtilis*, *Pseudomonas fluorescens,* and *Streptomyces* species were reported as commercial biocontrol agents for controlling *Phytophthora* species (BCA) [2, 19–25]. The mechanisms of parasitism, competition, antibiotic, and enzyme were performed in different antagonistic microorganisms.

The extracellular cell wall degrading enzymes including chitinases, cellulases, amylases, and 1, 3-β-glucanases are found in the genus *Streptomyces* [26–28]. The high chitinases and cellulases activities were produced by *S. rubrolavendulae* S4.

The 51.42 and 34.10 percentage of survival of tomato and chili seedling were observed in pots containing *P. infestans*, respectively (Table 1). After 3–5 days of cultivation in pots, the tomato and chili appeared to contain seedling damping off disease. A soften, decayed, weakened, and die seedling were expressed in pregerminated and postgerminated chili and tomato seeds. Then, peat mosses or seeds were transferred onto PDA plates and cultured at room temperature for 3 days (Figure 4). After that the similarity characteristics to *Phytophthora infestans* were observed from that plate. An aseptate mycelium, lemon shape of sporangium, and oogonium were observed under a light microscope (10X). The results indicated that the expression of damping off disease in chili and tomato seedlings is caused by *Phytophthora infestans*. After 50 days of cultivation, the healthy tomato and chili transplants were performed in treatments with added *S. rubrolavendulae* S4. These transplants showed higher height and weight than other treatments (data not show) (Figure 5). The bioactive natural compounds and plant hormones can be produced from several *Streptomyces* species and affect the host plant as plant growth promotion bacteria [29, 30]. The results clearly demonstrated that seedling damping off disease of tomato and chili in economic plant nurseries can be controlled by *S. rubrolavendulae* S4.

5. Conclusion

The chitinolytic *S. rubrolavendulae* S4 had a strong-antagonistic activity against *Phytophthora infestans*, isolated from the infected tomato plant seedlings. Therefore, *S. rubrolavendulae* S4 can be used as a good biocontrol for seedling damping off disease in economic plant nurseries. In *P. infestans* contaminated peat moss, the biocontrol increased the percentage of surviving tomato and chili seedling from 51.42 to 88.57 and 34.10 to 76.71, respectively. The mass cell production of *S. rubrolavendulae* S4 in an appropriate medium will be conducted as future work.

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