CHAPTER 4
ANTAGONISTIC MECHANISMS OF *STREPTOMYCES* SP. STRAIN S4 AGAINST SEEDLING DAMPING OFF FUNGI

4.1. Abstract

Antagonistic mechanisms of *Streptomyces* sp. strain S4 against seedling damping off fungi were investigated. The growth inhibition on solid agar and in broth culture of derived from hydrolytic enzyme activities or/and antifungal substance produced by the antagonist. Chitinase production in colloidal chitin medium and cellulose production in carboxyl methyl cellulose (CMC) medium were studied. The highest extracellular chitinase and cellulase were generated in 5 days at 12.1 and 65.0 Units enzyme/ml, respectively. The extracellular enzymes in the antagonistic culture supernatant were inactivated by heat. The heated and non-heated antagonistic culture supernatants were tested for growth inhibition of *P. infestans* and *P. aphanidermatum* by agar well diffusion method. Heated supernatant had the fungal growth inhibitory effect less than non-heated supernatant. Therefore, the fungal growth was inhibition by both extracellular hydrolytic enzymes and antifungal substance. For cell structure of both pathogenic fungi was mainly composted of β-glucan, the fungal mycelium destruction by the antagonist was testified. The parasitic mechanism of the antagonistic *Streptomyces* sp. strain S4 against, *P. infestans* and *P. aphanidermatum* was demonstrated clearly by scanning electron micrographs. The antagonistic *Streptomyces* sp. strain S4 protection of tomato and chinese spinach seedlings from damping off disease caused by *P. infestans* and *P. aphanidermatum* were confirmed.

Keywords : Antibiosis / Parasitism / Scanning Electron Microscopy

4.2. Introduction

Biological disease control is an interesting alternative for controlling of plant diseases. Mechanisms of biological disease control are interactions of antagonists to pathogens. For successful biocontrol, the biological control agents are selected and developed to be the effective biocontrol agents in the suitable environment. The antibiosis, competition, mycoparasitism, cell wall degrading enzymes, and induced resistance are mechanisms

of biological control. In general, one or more than one mechanisms are generated by antagonistic microorganisms in biocontrol process.

Streptomyces has been known as the largest antibiotic-producing genus of the microbial population in soil. They usually produce secondary metabolites as antibiotic and/or enzyme during sporulation (Challis and Hopwood 2003; Ahmed et al., 2013). Many antibiotics were produced by streptomycetes and other related family such as aminoglycosides, polyether, tetracyclines, macrolides,β-lactams, peptides, and polyenes (Liu et al., 2013; Omura et al.,1984). In recent study the different functions of these compounds can act as biological control in agriculture (Strobel et al., 2004; Arasu et al., 2013). Furthermore, *Streptomyces* also produce hydrolytic enzymes for degrading various polymers in environment such as lignocelluloses, chitin, and pectin (Crawford 1978; Sandgren et al., 2005; Chater et al., 2010; Eijsink et al., 2010). The biological control agent, *Streptomyces* has been reported in for their different mechanisms against several plant pathogens such as *P. porphyrae* (Woo et al., 2002), *F. oxysporum* (Getha and Vikineswary, 2002), and *Phytophthora* spp. (Fayad et al., 2001).

The antibiosis is known as the antagonism resulting from metabolites is toxic to pathogen. *Pseudomonas* spp., *Bacillus* spp., *Streptomyces* spp. and *Trichoderma* spp. are commonly found as antibiotic producer. They can be produced several type of antibiotic compounds which having different activities and targets. The mechanism of antibiosis was antibiotic compound releasing or diffusing rapidly into the environment. According to many studies, antibiotics were produced and released in soil such as geldanamicin and nigericin from *S. violaceusniger* YCED9, Antimycin A from *S. albidoflavus*, 6-Prenylindole from *Streptomyces* sp. TP-A0595, and Saadamycin from *Streptomyces* sp. HEDAYA48 (Trejo-Estrada et al., 1998; Sasaki et al., 2002; El-Gendy and El-Bondkly, 2010; Yan et al., 2010). In addition, another mechanism of biocontrol was parasitism which reducing fungal pathogens by antagonist extracellular hydrolytic enzymes (Picard et al., 2000; Whipps, 2001). Hydrolytic enzymes, β -1, 3 glucanases, cellulases and chitinases played a role in fungal cell walls degradation and predation (Masih and Paul, 2002; Matroodi et al., 2013; Chavan and Deshpande, 2013; Phitsuwan et al., 2013). *Trichoderma* spp., a well-known biocontrol agent, is usually coexisted with plant pathogen for a prolonged period of time by attaching and coiling mycelium around the pathogen. Then cell wall-degrading enzymes and active compounds were

produced and used to entry mycelium tip into the pathogen hyphae. *T. harzianum*, another biocontrol agent, is parasite to *F. oxysporum, P. ultimum, R. solani, S. sclerotiorum,* and *S. rolfsii*, it coiled its mycelium around the pathogen and followed by penetrated into the pathogen hyphae (Benhamou and Chet, 1993; 1996; 1997; Qualhato et al., 2013). The ability of antibiotic and/or extracellular enzyme production and colonization of *Streptomyces* spp. is ideally suited for being used as biocontrol agent. The biological control mechanism was investigated in this chapter. The ability of antibiosis and mycoparasitism of *Streptomyces* sp. strain S4 against *P. infestans* and *P. aphanidermatum* was studied. The control of the damping off disease by *Streptomyces* sp. strain S4 was also determined.

4.3. Materials and Methods

4.3.1 Production of Extracellular Hydrolytic Enzymes

Streptomyces sp. strain S4, a hydrolytic enzyme producing actinomycetes was isolated from a termite mound in Kanchanaburi province, Thailand. From hydrolytic enzyme testing in Chapter 3 showed that *Streptomyces* sp. strain S4 produced the extracellular chitinase and cellulase. *Streptomyces* sp. strain S4 was maintained on nutrient agar plate at room temperature.

1. Chitinase Production of *Streptomyces* sp. Strain S4 in Colloidal Chitin Medium

Streptomyces sp. strain S4 maintained on colloidal chitin agar was pre-cultured in colloidal chitin broth for 48 hour at 30°C under shaking conditions (150rpm) as starter ($1x10^6$ CFU/ml). The 250ml of Erlenmeyer flask containing 50 ml of 1% colloidal chitin medium (pH 7) were prepared. A 0.5 ml of starter was transferred into 50 ml of colloidal chitin broth. The liquid medium was incubated at 30°C for 7 days under shaking conditions. The growth and chitinase activity of *Streptomyces* sp. strain S4 was measured daily by using drop plate method and the p-dimethyl amino benzaldehyde (DMAB) method, respectively (Appendix D).

2. Cellulase Production of *Streptomyces* sp. Strain S4 in CMC Medium

Two days culture of *Streptomyces* sp. strain S4 in CMC broth was used as starter for growth and cellulase production experiments. A 0.5 ml of cell suspension was

transferred into 50 ml of CMC culture broth (pH 6) in a 250 ml Erlenmeyer flask and incubated at 30°C in rotary shaker at 200 rpm for 7 days. The growth and cellulase activity were measured daily by using a drop plate method (Herigstad et al., 2001) and dinitrosalicylic acid (DNS) method (Miller, 1959), respectively (Appendix D).

3. Chitinase and Cellulase Activities of *Streptomyces* sp. Strain S4 in Solid Medium

The solid medium consisted of 5g shrimp shell powder and 5g rice bran was prepared in 250-mL Erlenmeyer flask. The medium was autoclaved at 121 °C 15lbs/m^2. *Streptomyces* sp. strain S4 was pre-cultured in basal mineral salts broth (Appendix B) with 1% shrimp head for 48 hour at 37°C under shaking conditions (200 rpm) as starter ($1x10^6$ CFU/ml). The solid culture was incubated at room temperature for 9 days. During incubation, the growth was monitored by using the serial dilution standard plate-count technique (Jorgensen et al., 1979). Dinitrosalicylic acid (DNS) method was used for measuring chitinase and cellulase activities (Miller, 1959).

4.3.2 Antagonistic Test of *Streptomyces* sp. Strain S4 Against *Phytophthora infestans* and *Pythium aphanidermatum*

1. Dual Culture Method

The pathogen-antagonist interaction was observed by using a dual culture technique on a solid medium. The *Streptomyces* sp. strain S4 was streaked on PDA at the distance of 2.0 cm from the edge of the Petri dish. The plates were then incubated at 30°C for 3 days to allow growth and sporulation of the *Streptomyces* sp. strain S4. Afterwards 4 mm diameter freshly-growing mycelium plug of fungal pathogens, *P. infestans* and *P. aphanidermatum* was placed away from strain S4 lined (4.0 cm) and incubated for another 3 days. The pathogen alone was used as a positive control and the experiment was repeated three times. The non-fungal growth area surrounding the *Streptomyces* sp. strain S4 culture indicated antagonistic activity. Percentage of growth inhibition was determined by this formula of Skidmore (Skidmore, 1976).

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

Where R represents the radius of the control pathogen growth, and r the radius of the pathogen's growth towards the bacterial antagonist

2. Fungal Growth Inhibition in Liquid Medium

A co-cultured method was used to examine the inhibition of fungal growth in a liquid medium. The *Streptomyces* sp. strain S4 was cultured in CMC broth at 30 °C for 2 days (10^4 CFU/ml). Then 1.0 ml of suspension was added into 20.0 ml of PDB which contained two mycelium plugs of fungal pathogen. The PDB without *Streptomyces* sp. strain S4 was used as a control. After 3 days incubation, the dry weight of the mycelium was determined in an oven at 60°C for 6 hours. The experiments were repeated three times with three replications for each experiment.

4.3.3 Evaluation of Biological Control Mechanisms of *Streptomyces* sp. Strain S4

1. The Agar Well Diffusion Method

The heat-inactivated extracellular enzyme of *Streptomyces* sp. strain S4 was done by heating the supernatant of 5 days culture in CMC medium and colloidal chitin medium at 100°C for 10 min. The experiment was performed under strictly aseptic conditions. Wells were made in PDA plates with a sterile borer (4 mm diameter) and a mycelium plug of *P. aphanidermatum* was placed on the center of the plate. A heated or non-heated supernatant sample of *Streptomyces* sp. strain S4 (200 µl) was introduced into the wells. Sterile distilled water was used as a control. The plates were incubated at 37°C for 2 days. All samples were tested in triplicate. Inhibition of fungal growth was determined.

2. Light and Scanning Electron Microscopy Investigation on Interaction between *Streptomyces* sp. Strain S4, *P. infestans*, and *P. aphanidermatum*

The mycoparasitism of *P. aphanidermatum* cell wall by *Streptomyces* sp. strain S4 was studied using light microscopy (CHS; Olympus optical Co. Ltd, Japan) and scanning electron microscopy (JEOL JSM-6610CV, Japan). Spores of *P. aphanidermatum* and the conidia of *Streptomyces* sp. strain S4 were inoculated into a warm melted PDA drop on a sterile cover glass in a culture chamber incubated at room temperature for 3-5 days and then observed by light microscopy. The co-cultures on the cover glasses were prepared and examined by scanning electron microscopy (JEOL JSM-6610CV, Japan).

4.3.4 Biological Activity of Antagonistic *Streptomyces* sp. Strain S4 to *P. infestans* and *P. aphanidermatum*

1. Evaluating Biocontrol of Damping Off Disease of Tomato and Chili Seedling

Peat mosses was sterilized for 15 minutes at 121°C 15 lbs/in^2 three times at 24h intervals and used as a planting material in this study. The agar plugs, took 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. The *Streptomyces* sp. strain 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 *Streptomyces* sp. strain S4 cell suspension was inoculated into the peat moss at the final concentration of 10^6cfu/g. The experimental design was a 2x5 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 *Streptomyces* sp. strain S4, 3. artificial fungal pathogen infested but treated with fungicide, metalaxyl (Phyto-Q), *Streptomyces* sp. strain S4 inoculated, and 4. non-inoculated planting material as control. Percentages of the non-infested seedling were then determined.

2. Evaluating Biocontrol of Damping Off Disease of Chinese Spinach Seedling

The experiment was a 2 x 3 completely randomized factorial design with three replicates. Sterilized peat moss was used the growing medium in this study. The growing medium was prepared in two ways: artificially infested and non-infested with *P. aphanidermatum.* Infestation of peat moss with *P. aphanidermatum* was achieved by adding 50 plugs (4 mm diameter) of freshly-grown *P. aphanidermatum* into 250g of steam-sterilized peat moss. Each preparation of the peat moss was separated into three treatments: 1) No pathogenic fungi, 2) *Streptomyces* sp. strain S4 biocontrol treatment, and 3) Metalaxyl (Phyto-Q) fungicide treatment. The *Streptomyces* sp. strain S4 biocontrol was applied at the rate of 10^6 CFU/g of peat mosses. Phyto-Q was used at the rate of 0.5g/l according to the manufacturer's guidelines. Three replicates, each of 50 Chinese spinach seedlings were grown in the 6 treatments for 10 days. The percentage of disease suppression was calculated by counting the number of non-infested seedlings.

4.4. Results and Discussions

4.4.1 Production of Extracellular Hydrolytic Enzymes

1. Chitinase Production of *Streptomyces* sp. Strain S4 in Liquid Medium

The liquid batch fermentation is commonly used for enzyme production in various substrates. Growth and chitinase production of *Streptomyces* sp. strain S4 in colloidal chitin medium were maximized at 7.68 Log CFU/ml and 12.1 U/ml, respectively in day 5 (Figure 4.1). The results clearly showed that both of enzyme and cell number were increased as the induction time increased. According to Kim (2001) report, *S. griseus* HUT 6037 produced the maximum specific activity of chitinase (12.6 U/mg proteins) in medium contained colloidal chitin as a carbon source on the day 5 of cultivation. Several authors have reported that chitinase activities of *Serratia marcescens* XJ-01 could reach 15.6 U/ml at optimal culture conditions (Xia et al., 2011).

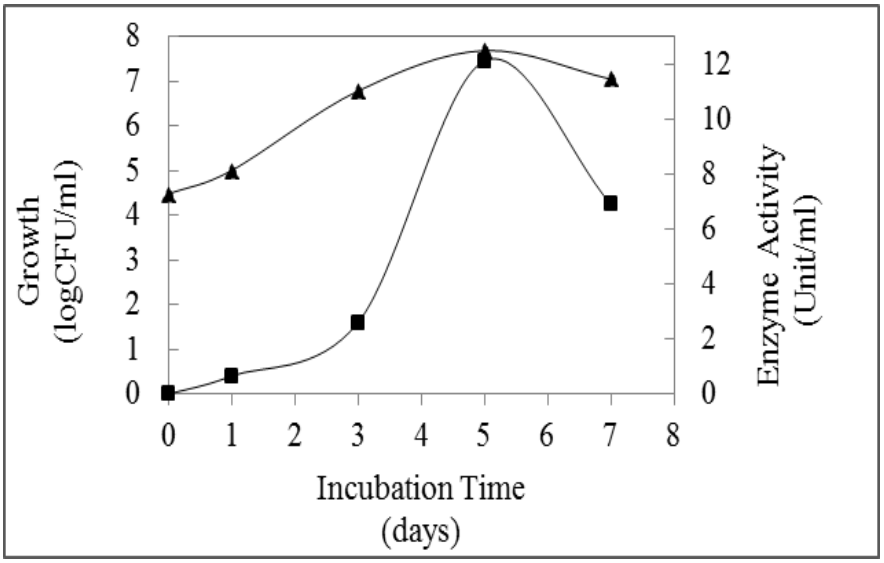

Figure 4.1 The growth and chitinase activity of *Streptomyces* sp. strain S4 cultured in colloidal chitin medium. (filled quare) chitinase activity, (filled triangle) growth.

2. Cellulase Activity of *Streptomyces* sp. Strain S4 in Liquid Medium

The genus *Streptomyces* are usually produced enzymes and secondary metabolites which can utilize a wide range of organic compounds. Growth and cellulase production of *Streptomyces* sp. strain S4 in CMC medium were maximized at 7.85 logCFU/ml and 65.21 U/ml, respectively in day 5 (Figure 4.2). Enzyme production increased as growth

increased. Since the major component of oomycetes cell wall is cellulose (Manocha and Colvin, 1968; Inglis and Kawchuk, 2002), the extracellular cellulase of *Streptomyces* sp. strain S4 could have potential for destroying fungal mycelium.

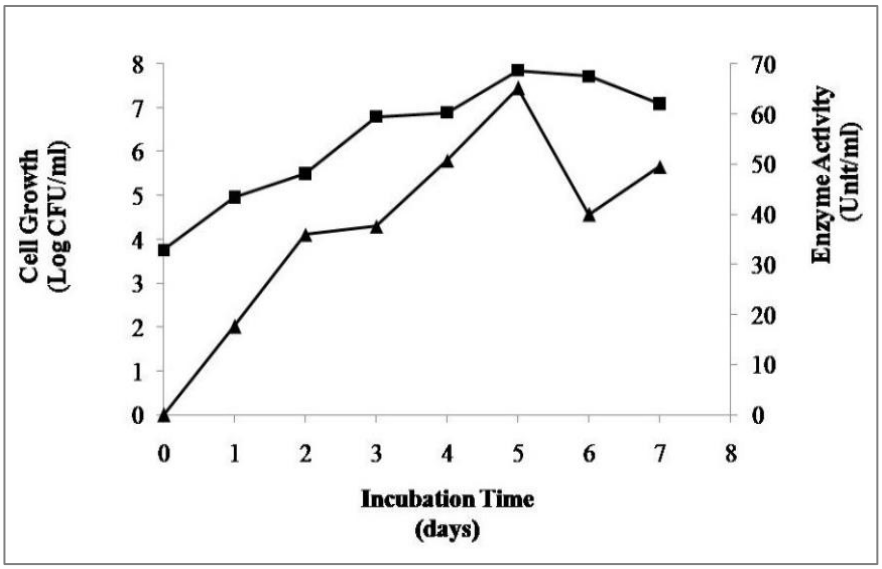

Figure 4.2 The growth and cellulase activity of *Streptomyces* sp. strain S4 cultured in CMC medium. (filled square) growth, (filled triangle) cellulase activity.

3. Chitinase and Cellulase Activities of *Streptomyces* sp. Strain S4 in Solid Medium

Solid-state fermentation is an attractive method for microbial cell production of *Streptomyces* sp. strain S4 in solid-state fermentation medium contained shrimp shell powder, 5g; rice brain, 5g; moisture level, 60% was highest at 8.11logCFU/g dry matter in day 3 (Figure 4.3). In addition, chitinase and cellulase production of strain S4 were maximized in day 5 at 11.5 U/g dry matters and 128.17 U/g dry matter, respectively.

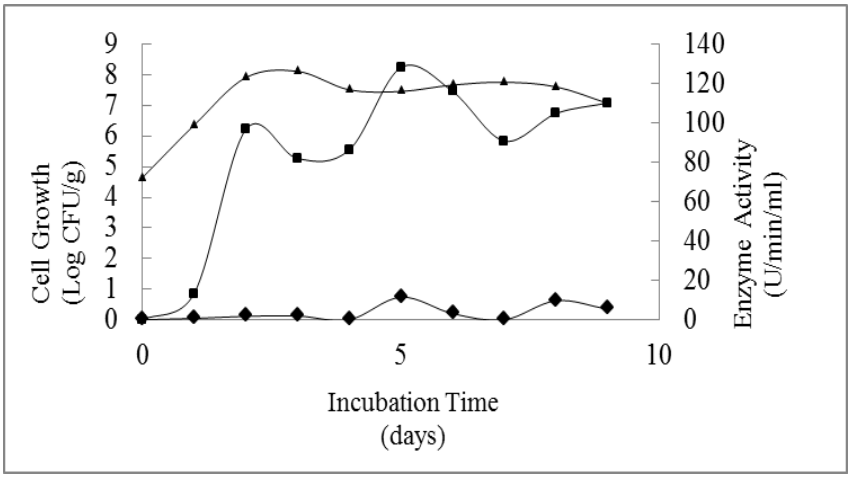

Figure 4.3 The growth, chitinase and cellulase production of *Streptomyces* sp. strain S4 in solid-state fermentation medium. (filled triangle) growth, (filled square) cellulase activity, (filled diamond) chitinase activity.

4.4.2 Antagonistic Activity of *Streptomyces* sp. Strain S4 Against *P. infestans* and *P. aphanidermatum*

The dual culture method is commonly used to investigate the growth inhibition interaction between two cultures. Growth inhibition of *P. infestans* and *P. aphanidermatum* by strain S4 were demonstrated in Figure 4.4 and Figure 4.5. *Streptomyces* sp. strain S4 could inhibit growth of *P. infestans* better than *P. aphanidermatum.*

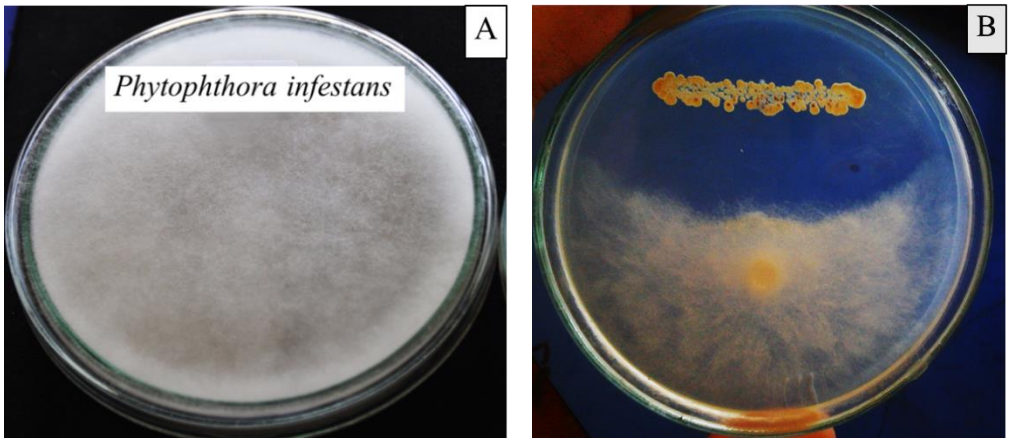

Figure 4.4 Photographs of *in vitro* interactions between *Streptomyces* sp. strain S4 and *P. infestans* in a dual culture on PDA. Control plate of *P. infestans* (A), *Streptomyces* sp. strain S4 against *P. infestans* (B).

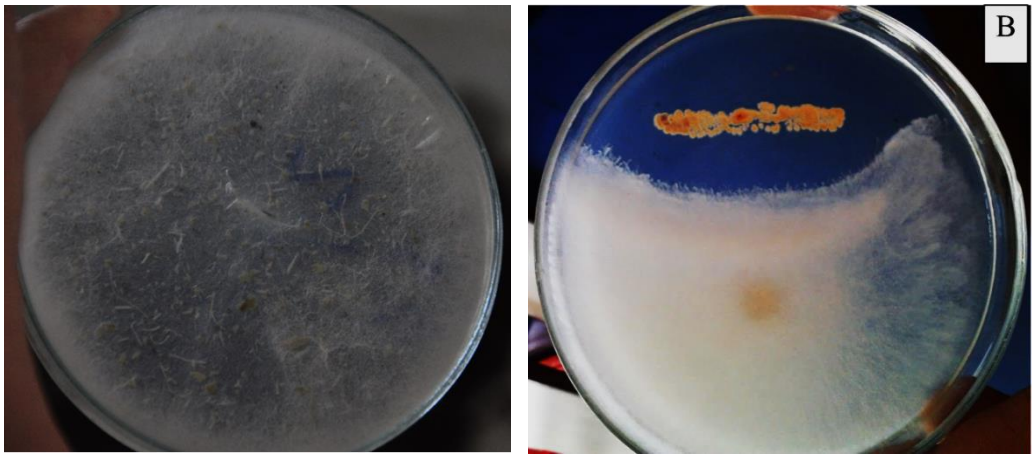

Figure 4.5 Photographs of *in vitro* interactions between *Streptomyces* sp. strain S4 and *P. aphanidermatum* in a dual culture on PDA. Control plate of *P. aphanidermatum* (A), *Streptomyces* sp. strain S4 against *P. aphanidermatum* (B).

The dry weight of *P. infestans* and *P. aphanidermatum* cell mass cultured for 3 days in PDB were 78.23mg/20ml and 70.4mg/20ml, respectively. When the pathogenic fungi was co-culture with *Streptomyces* sp. strain S4, the cell mass of *P. infestans* and *P. aphanidermatum* was only at 13.4mg/20ml and 28.47mg/20ml, respectively. Therefore, *Streptomyces* sp. strain S4 could suppress the growth of both pathogens in submerge culture. Similarly in previous reports, the pathogen structures were degraded by the cell wall lytic enzymes and mycelium growth was limited by the activity of the antagonist (Gupta et al., 2001).

Table 4.1 Ability of *Streptomyces* sp. strain S4 to inhibit mycelial growth of fungal pathogens in liquid medium

Treatment	**Average Mycelium Dry Weight (mg /20 ml medium)**	
	P. infestans	***P. aphanidermatum***
PDB	78.23	70.4
PDB with Strain S4	13.4	28.7

* The value of each treatment is the average dry weight of three mycelium mats.

4.4.3 Mechanisms of Antagonistic Activity

1. Antibiosis Mechanisms

Since the fungal cell walls was typically chitin and/or glucan, *Streptomyces* sp. strain S4 was cultured in media containing colloidal chitin and CMC as enzyme inducer. Fungal growth inhibition by antifungal compounds and/or hydrolytic enzymes produced by the antagonist was tested using heat and non-heat inactivated culture supernatants of the antagonist in liquid media. Both supernatants showed similar inhibitory effect on fungal growth (Figure 4.4-4.5). The extracellular hydrolytic enzymes of *Streptomyces* sp. strain S4 should have been interacted by high heat treatment in sample preparation process. Heat interacted culture supernatant still gave fungal growth inhibition but at a lower effect than the non-heated culture supernatant. This should derive from the heat resistant antifungal substances. Therefore, the fungal growth inhibition should be obtained by both hydrolytic enzymes and antifungal substances produced by the antagonist.

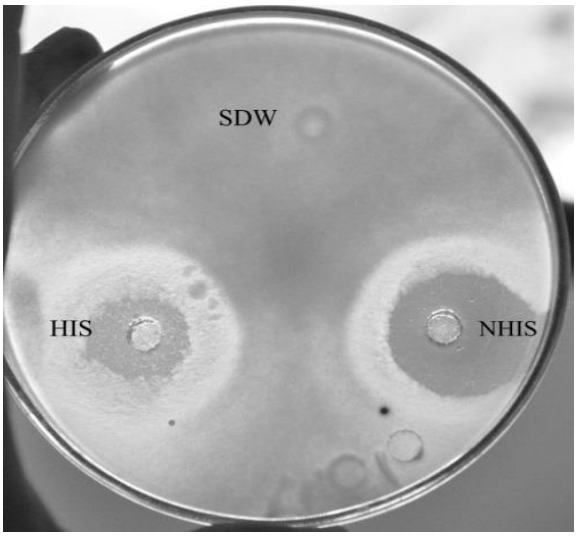

Figure 4.6 The heat and non-heat inactivated culture supernatant of *Streptomyces* sp. strain S4 in CMC medium showed inhibitory effect on *P. aphanidermatum* growth. (SDW) Sterile distilled water as a control, (HIS) Heat inactivated supernatant of Streptomyces sp. strain S4, (NHIS) Non-heat inactivated supernatant of *Streptomyces* sp. strain S4. Two days after incubated, *P. aphanidermatum* photographed from the bottom.

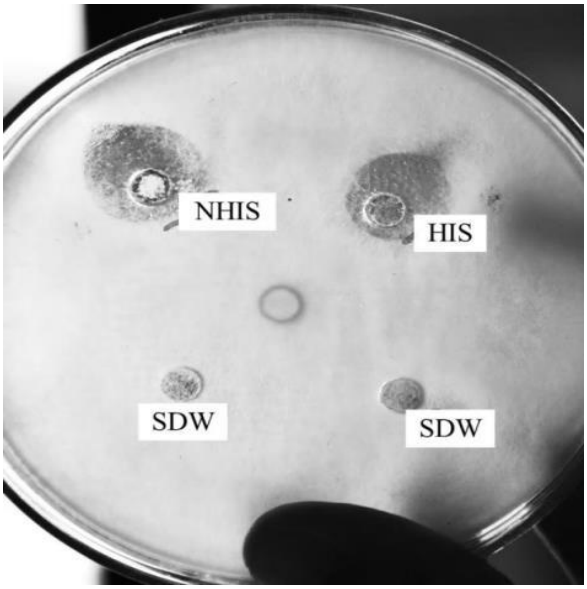

Figure 4.7 The heat and non-heat inactivated culture supernatant of *Streptomyces* sp. strain S4 in colloidal chitin medium showed inhibitory effect on *P. aphanidermatum* growth. (SDW) Sterile distilled water as a control, (HIS) Heat inactivated supernatant of *Streptomyces* sp. strain S4, (NHIS) Non-heat inactivated supernatant of *Streptomyces* sp. strain S4. Two days after incubated, *P. aphanidermatum* photographed from the bottom.

2. Mycoparasitism and Fungal Cell Wall Degradation by Antagonist

One of biological control mechanism, mycoparasitism is usually found in filamentous bacteria or fungal antagonist. The important interactions are occurred by the cell wall degrading enzymes. In this study, the penetration of mycelium of *Streptomyces* sp. strain S4 into the mycelium of *P. infestans* and *P. aphanidermatum* were revealed. The claiming small sized filamentous of strain S4 together with both of pathogens was observed by using SEM method. Scanning electron micrographs demonstrated that mycelium of *Streptomyces* sp. strain S4 were covered and attached nearby *P. infestans* (Figure 4.8) and *P. aphanidermatum* mycelium (Figure 4.9). The SEM results confirmed that *Streptomyces* sp. strain S4 had mycoparasitic ability to fungi. For clear result, the higher magnification of SEM was used to study the interaction of both the pathogen and the antagonist. The result showed that strain S4 was parasitized by branching, attaching, and growing parallel to *P. infestans* and *P. aphanidermatum*.

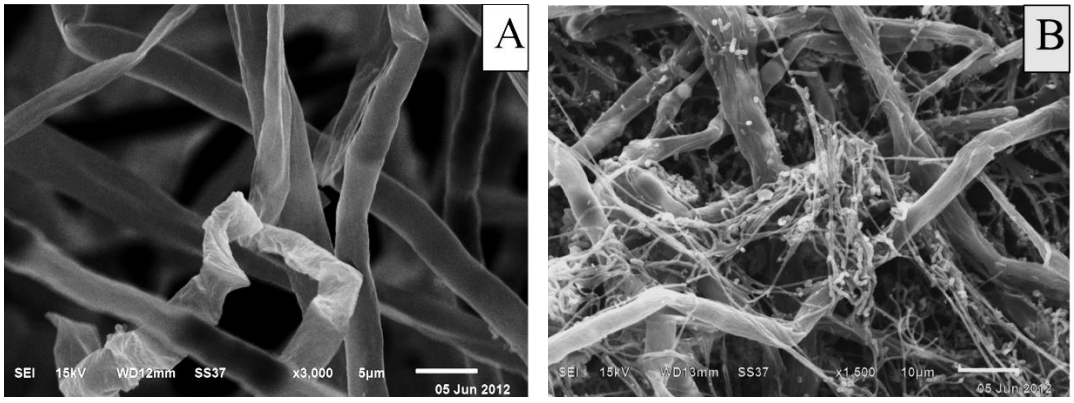

Figure 4.8 Scanning electron micrographs of the antagonist and *P. infestans* at 5 days co-cultured in PDA on cover glass. Mycelium of *P. infestans* (A), *Streptomyces* sp. strain S4 hyphae grow on mycelium of *P. infestans* (B).

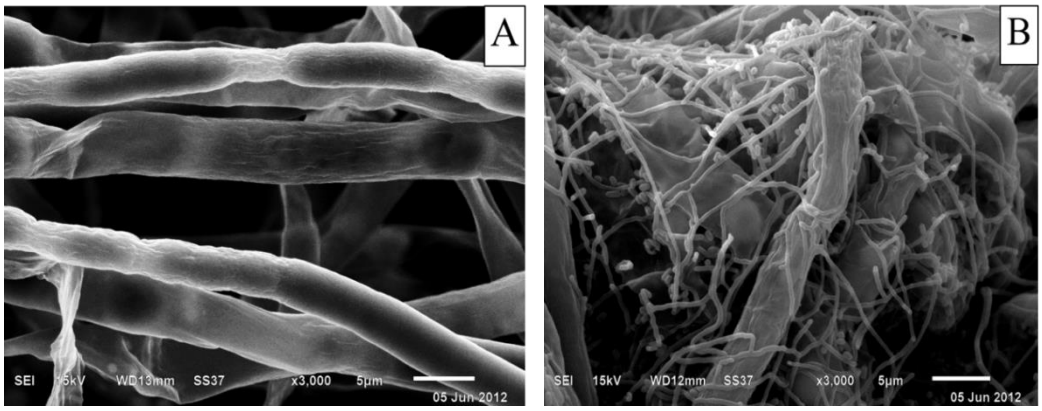

Figure 4.9 Scanning electron micrographs of the antagonist and *P. aphanidermatum* at 5 days co-cultured in PDA on cover glass. Mycelium of *P. aphanidermatum* (A), *Streptomyces* sp. strain S4 hyphae grow on mycelium of *P. aphanidermatum* (B).

During sporulation, genus *Streptomyces* is generally produced extracellular hydrolytic enzymes and antibiotics as the secondary metabolites. Therefore, many small pores on *P. aphanidermatum* mycelium were found at the locations that spores of S4 were presented (Figure 4.10). The deformation of mycelium could occur by the action of the cell wall degrading enzymes. Based on the previously finding that *Streptomyces* sp. strain S4 produced cellulase and chitinase. Since oomycetes mycelium was composted of cellulose and glucan, the mycelium structure might be destroyed by the strain S4 cellulase. It is clear that antagonistic *Streptomyces* sp. strain S4 had two mechanisms; antibiosis and mycoparasitism.

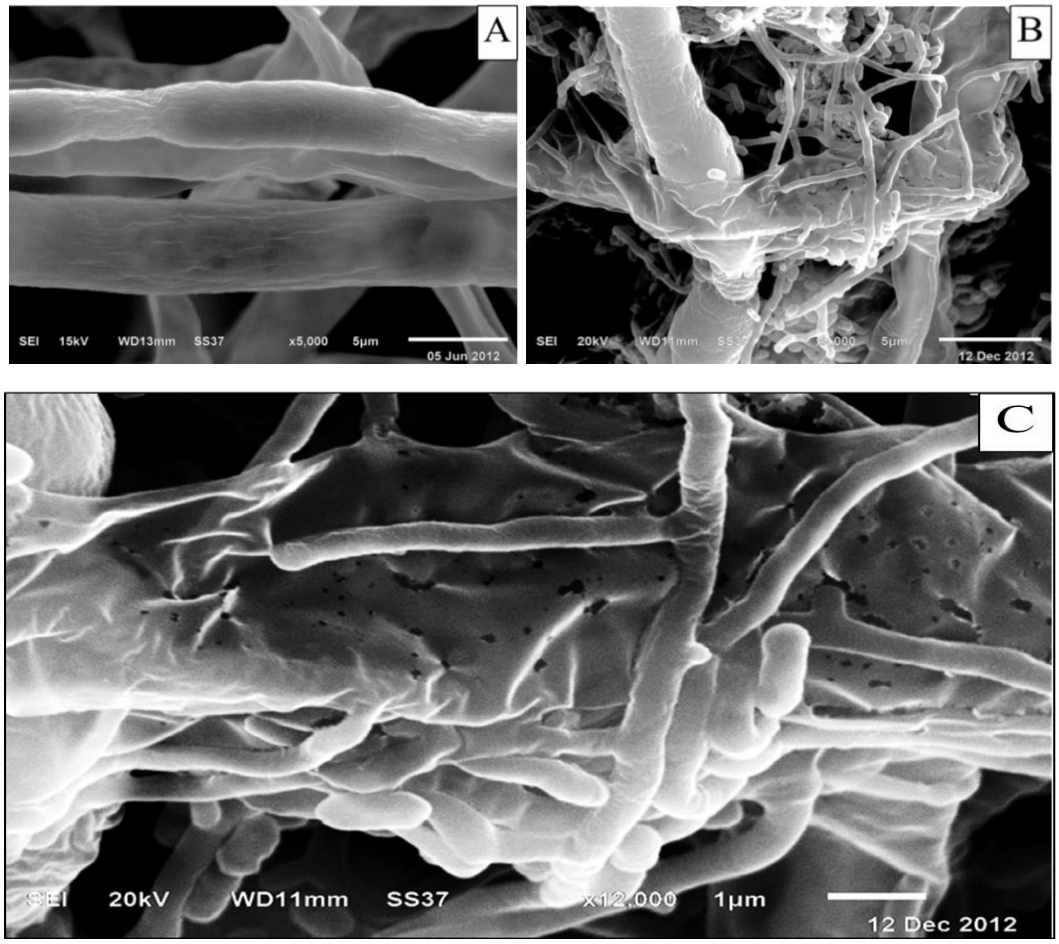

Figure 4.10 Scanning electron micrographs of the deformation of the fungal mycelium by the antagonist at 5 days after co-cultured. Normal mycelium of *P. aphanidermatum* (A), The porous *Streptomyces* on *P. aphanidermatum* mycelium (B,C).

4.4.4 Biological Control Activity of Antagonistic *Streptomyces* sp. strain S4 against *P. infestans* and *P. aphanidermatum*

1. Damping Off Disease Suppression in Tomato and Chili Seedling

Many chemical fungicides are usually applied in the plant fields, greenhouses and nurseries for disease management. Actually, the biological control agent can be applied to manage plant disease for producing good health and safe environment. Symptoms of damping off disease were observed within 3-5 days of tomato and chili seedlings being planted into the pathogen antifungicide inoculated peat moss (Table 4.2). In *P. infestans* contaminated peat moss, the survival of tomato and chili seedlings was significantly increased ($p<0.05$) from 51.42 to 88.57% and 34.10 to 76.71% for the strain S4 treatment, respectively. The *Streptomyces* sp. strain S4 also showed high efficiency

equivalence to fungicide, metalaxyl with no significant difference (P≥0.05). The results clearly demonstrated that seedling damping-off disease of tomato and chili in Economic Plant Nurseries could be controlled by *Streptomyces* sp. strain S4. Metalaxyl and fosetyl-A1 are normally suggested chemical fungicides to be used against *Phytophthora* species. They are dangerous for the environment (Edington et al., 1971; Ioannou and Grogan, 1984; Davis, 1989; Flett et al., 1991). Pugliese (2010) reported that *Phytophthora* root rot could be controlled by infesting peat moss with *Streptomyces* at the time of planting under greenhouse conditions.

Table 4.2 Biological and chemical control of tomato and chili damping off disease caused by *P. infestans*.

Treatment	% non-infested tomato seedling	% non-infested chili seedling
Control	88.56[b]	95.71[c]
P. infestans	51.42[a]	34.10[a]
P. infestans+ Strain S4	88.57[b]	76.71[b]
P. infestans + Phyto-Q	94.28[b]	79.99[b]
Strain 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).

2. Damping Off Disease Suppression in Chinese Spinach Seedling

Chinese spinach seedlings are traditionally produced in plastic pots which use peat moss as a planting material. It takes only 7-10 days before the seedling are sold to customers. Chemical fungicide treatments are not suited for this short time of cultivation, since the residues of these hazardous chemicals can remain on the edible parts of the plants. Therefore, biological control methods would be a better alternative approach under such production methods. Symptoms of damping off were observed within 5-10 days of the Chinese spinach seedlings being planted into the pathogen inoculated peat moss (growing medium). In the sterile growing medium, the proportion of healthy, non-infected seedlings was greater than 82% in all treatments. When seedlings were grown on peat moss contaminated with *P. aphanidermatum*, the proportion of non-infected

seedlings was only 46.9% which was significantly different from the control treatment (87.6%). Treatment with *Streptomyces* sp. strain S4, as a biological control agent, markedly suppressed damping off disease to levels similar to those achieved with the control and with the fungicide treatment. These results indicate that *Streptomyces* sp. strain S4 is an effective biocontrol against *P. aphanidermatum*. In the plant protection experiment reports here, the antagonistic *Streptomyces* sp. strain S4 protected seedlings from damping off disease successfully when the antagonist was colonized in the planting material before artificial infection of *P. aphanidermatum*.

Table 4.3 Biological and chemical control of chinese spinach damping off disease caused by *P. aphanidermatum*.

Treatment	% Non-infected seedling
Non-infected peat moss	
Control	87.55[b]
Streptomyces sp. strain S4	81.77[b]
Phyto-Q	94.00[b]
Infected peat moss (*P. aphanidermatum*)	
P. aphanidermatum	46.88[a]
P. aphanidermatum + *Streptomyces* sp. strain S4	74.00[b]
P. aphanidermatum + Phyto-Q	81.33[b]

[a, b] Means within a column with the same letter were not significantly different when $p \geq 0.05$.

4.5. Conclusion

The antagonistic *Streptomyces* sp. strain S4 could inhibit mycelium growth of *P. infestans* and *P. aphanidermatum* in both of solid and submerge medium. *Streptomyces* sp. strain S4 could grow and produce hydrolytic enzymes, chitinase in colloidal chitin medium and cellulose in carboxyl methyl cellulose (CMC) medium. Both of hydrolytic enzymes and antifungal substances of the antagonist *Streptomyces* sp. strain S4 could against *P. infestans* and *P. aphanidermatum*. The photograph of SEM is clearly demonstrated the mycoparasitism of strain S4 to *P. infestans* and *P. aphanidermatum*. The seedling damping off disease of tomato, chili, and chinese spinach in Economic Plant Nurseries could control by *Streptomyces* sp. strain S4.