CHAPTER 6 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The seedling damping off disease fungi could cause the serious problem in economic plant nurseries in Thailand. The infected tomato plant seedling was obtained from an economic plant nursery in Amphoe Pak chong, NaKhon Ratchasima Province, Thailand. The pathogenic fungi could identify to be *Phytophthora infestans* based on the morphological characteristic. The damping off Chinese spinach seedling was obtained from an economic plant nursery in Chiang Mai Province, Thailand. The pathogenic fungi was identified to be *Pythium aphanidermatum* based on the morphological characteristic. Therefore, *P. infestans* and *P. aphanidermatum* were causative agents of the seedling damping off disease.

Among six soil actinomycetes isolates, isolated from termite mounds at the grove of Amphoe Si-sawat, Kanchanaburi Province, Thailand, *Streptomyces* sp. strain S4 had an antagonistic effect against both both *P. infestans* and *P. aphanidermatum*. The isolate S4 could classify into the genus *Streptomyces* based on the micrograph of spore chains and 16S rRNA gene sequence. The genotypic and phenotypic data of the strain S4 is clearly differentiated from the reference strains, *Streptomyces fradiae* and *S. rubrolavendulae* which had 100% similarity of 16S rRNA. Therefore, we will propose this strain to be a new species of genus *Streptomyces.*

The biological control activity of genus *Streptomyces* has been reported with different mechanisms against several plant pathogens. The growth inhibition on solid agar and in broth culture of *P. infestans* and *P. aphanidermatum* by *Streptomyces* strain S4 was observed. Heat and non-heat inactivated culture supernatant of *Streptomyces* strain S4 exhibited growth inhibition of both *P. infestans* and *P. aphanidermatum*. Therefore, the fungal growth was inhibited by both heat sensitive extracellular hydrolytic enzymes and heat tolerant antifungal substance produced by *Streptomyces* strain S4. For cell structure of both pathogenic fungi was mainly composed of β-glucan, the fungal mycelium destruction by the antagonist was testified. The parasitic mechanism of the antagonistic *Streptomyces* sp. strain S4 against *P. aphanidermatum* was demonstrated clearly by

scanning electron micrographs. It is clear that antagonistic mechanisms of *Streptomyces* sp. strain S4 against seedling damping off fungi were antibiosis and mycoparasitism.

The antagonistic *Streptomyces* sp. strain S4 protection of tomato and chinese spinash seedlings from damping off disease caused by *P. infestans* and *P. aphanidermatum* were determined. In pot experiment, the antagonistic *Streptomyces* sp. strain S4 could protect the tomato, chili, and chinese spinach seedlings from damping off disease. Adding *Streptomyces* sp. strain S4 into *P. infestans* contaminated peat moss could increase the survival of tomato and chili seedlings from 51.42 to 88.57% and 34.10 to 76.71%, respectively. These results were similarity with biological control of chinese spinach damping off disease caused by *P. aphanidermatum*. Mixing *Streptomyces* sp. strain S4 with peat moss contaminated with *P. aphanidermatum* could increase the proportion of non-infected seedlings from 46.9 to 74.0%. Moreover, Seedling in peat moss contaminated with *Streptomyces* sp. strain S4 could grow well with no significant difference to the control and the fungicide treatment ($P \geq 0.05$). Therefore, *Streptomyces* sp. strain S4 could apply in Economic Plant Nurseries as biological control agent.

The biological control *Streptomyces* sp. strain S4 cell mass could be produced by solid state fermentation. Since *Streptomyces* sp. strain S4 produces chitinase or cellulase to release reducing sugars from chitin or cellulose, the raw materials used in the fermentation were selected from the agro-industrial wastes that having chitin or cellulose as the major components. Shrimp heads and rice bran are low price agro-industrial wastes and easily to find in Thailand, so they were selected for using as nitrogen and carbon sources of the culture medium. The fermentation media was designed and optimized by using the response surface methodology (RSM) based on the central composite design (2^3 CCD). The maximum cell production at 6.94 log cfu/g substrate was obtained from the fermentation medium composed of 50:50 ratio of shrimp shell and rice bran, 60% moisture, and 1.5% inoculum application.

The production of biological control *Streptomyces* sp. strain S4 cell product was operated using the optimized fermentation conditions. The three days fermented cell mass was air-dried and turned into powder by crushing in a blender. The biological control product in the powder form was packed into aluminum foil bags with vacuum seal. The product had the initial antagonist cell at 7.3 logCFU/g. The batch keeping at

4°C could maintain the cell numbers at this level up to 6 months, while the batch keeping at room temperature could retain the same cell numbers for only 4 months and followed with gradually decline. The efficacy of the *Streptomyces* sp. strain S4 biological control product for controlling the seedling damping off disease still existed even after 6 months storage. Therefore, *Streptomyces* sp. strain S4 could be produced and used as the biological control product for controlling the seedling damping off disease caused by *P. infestans* and *P. aphanidermatum.*

6.2 Recommendations

The chemical structure and functional properties of the antifungal compound of the antagonistic *Streptomyces* sp. strain S4 should be further studied. For the most effective use of this biological control product, the filed application should be experimented. The potential of using the antagonistic *Streptomyces* sp. strain S4 for controlling other plant diseases should be researched.