CHAPTER V
CONCLUSION

One hundred isolates of actinomycetes were isolated from soil samples collected from the shore of Samed Island, Rayong province. Based on morphological, cultural, physiological and biochemical characteristics, 80 of them were identified as *Streptomyces* and the remaining 20 were identified as *Micromonospora*. Fifty-five isolates of *Streptomyces* and 14 isolates of *Micromonospora* exhibited antimicrobial activities, and most of them showed the ability to inhibit *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, and *Candida albicans* ATCC 10231. Interestingly, a number of these isolates also showed inhibitory activity against *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Among these isolates, PC 4-3 showed good antimicrobial activity and was selected for the secondary metabolite fermentation.

Strain PC 4-3 formed unbranched aerial hyphae, spiral spore chains. The diameter of the spores is 0.5 μm and the spore surface is rugose. On YMA medium, aerial spore-mass color was white but becoming black at maturity and a light brown diffusible pigment was formed. It could grow in YMA with 2% 4%and 6% NaCl, at pH 4.0, 5.0, 7.0, 9.0 and 10.0; at 10°C, and 28°C but not at 45°C. This strain liquefied gelatin and coagulated skim milk but showed no reaction to the reduction of nitrate, production of H_2S, formation of melanin, hydrolysis of starch, chitin, and cellulose. Utilization of glucose, glycerol, L-arbinose, D-xylose, mannitol, fructose, sucrose, melibiose, rhamnose and raffinose was positive. This strain contained L-diaminopemelic acid in cell wall. It was identified as *Streptomyces*. Comparision of 16S rDNA sequencing and phylogenetic analysis of the strain PC 4-3 to *Streptomyces* sp. NRRL B-1865 showed the high similarity of 99.52% that supported its taxonomic position as *Streptomyces* strain.

Fermentation of *Streptomyces* sp. PC 4-3 in YM broth at pH 6.0, 7.0 and 8.0 was suitable for the antimicrobial production. The ethyl acetate extract from YM fermentation broth of this strain inhibited the growth of *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633 and *C. albicans* ATCC 10231.

Antimicrobial assay-guided fractionation of the ethyl acetate extract yielded 2 known ansamycins including geldanamycin and 17-*O*-demethylgeldanamycin with the yield of 2.24% w/w and 0.24% w/w based on the ethyl acetate extract, respectively. The chemical structures of the isolated compounds were elucidated through extensive analyses of UV, IR, MS, and NMR spectroscopic data and the comparison with literatures. Geldanamycin, FPC43001a exhibited antimicrobial activity against *S. aureus* ATCC 25923 and *C. albicans* ATCC 10231 with 12.2 mm and 20.0 mm zone of inhibition at the concentation of 500 μg/disc.