CHAPTER V

CONCLUSION

As part of our continuing investigation on bioactive substances, twelve strains of actinomycetes isolated from soils were collected and identified. PNK1-3, PNK1-5, TT2-9 and KN-6 were identified as *Streptomyces*, FLM-2 as *Kitasatospora*, MA-1, MA-2, JSM1-1, JSM1-3, MC5-1, MC7-1 and R1-1 as *Micromonospora* strains based on morphological, cultural, physiological and biochemical characteristics including chemotaxonomic properties. The selected strains, PNK1-3 and FLM-2 produced extensive branched substrate, the mature aerial mycelium bearing long spore chains. Spores are non-motile. Initially, colonies are relatively smooth surface, but later they develop a weft aerial mycelium that may appear powder or velvety. The colors of the substrate mycelium were grayish white to black. Chemotaxonomically, the phospholipids pattern contained in the cells are diphosphatidylglycerol, phosphatidylethanolamine, phosphtidylinositol, phosphtidylmanosides, but phosphatidylcholine is not detected. Their predominant cellular fatty acids were iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{15:0}$, $C_{17:0}$, and anteiso-$C_{17:0}$ (Type 3b). Mycolic acids were absent. The G+C contents of the DNA of PNK1-3 and FLM-2 were 72.9 and 73.1 mol%. They showed the same pattern of chemotaxonomic characteristics which were similar to those of members of the genus *Streptomyces* and *Kitasatospora*. The higher ratio of *meso*-DAP to LL-DAP was useful to distinguish PNK1-3 and FLM-2.

Phylogenetic analysis of the almost complete 16S rDNA sequences revealed that the PNK1-3 was placed within the clade of the genus *Streptomyces* and FLM-2 was *Kitasatospora*. Based on the 16S rDNA sequences the range 99.9% and some physiological and biochemical properties, PNK1-3 should be identified to *S. hygroscopicus*. The other strain FLM-2 showed 16S rDNA sequences similarities the range 97.1% with *Kitasatospora melanogena*. For further studies, FLM-2 strain should be hybridized with *K. melanogena*; therefore it is very probable that the FLM-2 is a newly found species. PNK1-3 was isolated from soil in Patthaloong and FLM-2 from Pitsanuloak.

The remaining seven strains were *Micromonospora*. They produced well-developed and branched substrate hyphae but no aerial hyphae. Spores were borne singly on the substrate hyphae. Their spores were smooth, rough and nodular on the surface and non-motile. The colours of the substrate mycelium were brown to strong orange. All strains showed the same

pattern of chemotaxonomic characteristics which were similar to those of members of the genus *Micromonospora*. In general, the cell walls of *Micromonospora* are peptidoglycan type A1γ'. The acyl type of cell wall muramic acid was glycolyl. Cell wall hydrolysates contained glutamic acid, glycine, alanine, and diaminopimelic acid, and the isomer of diaminopimelic acid was meso, indicating that these strains have cell wall chemotype II. They contained glucose, xylose, arabinose, galactose, mannose and ribose as whole cell sugars (pattern D) but rhamnose was absent. The pattern of phospholipids and fatty acid corresponded to phospholipid type II and fatty acid type3b. The predominant menaquinones were MK-9(H_4), MK-10(H_4) or MK-9(H_6). The range of G+C contents of the DNA were from 71.9 to 72.9 mol%. Phylogenetic analysis of the almost complete 16S rDNA sequences revealed that the strains were placed within the clade of the genus *Micromonospora*. Based on the DNA-DNA relatedness, 16S rDNA and some physiological and biochemical properties, all strains could be separated into three groups. They were recognized as new species of *Micromonospora*. In this study, the names *Micromonospora krabiensis* sp. nov., *Micromonospora marinus* sp. nov., and *Micromonospora chaiyaphumensis* sp. nov. are proposed for Group I (2 strains), group II (2 strains) and Group III (3 strains), respectively.

As a result of this study, three new species of *Micromonospora* were found. They possessed an antimicrobial property, which can be of remarkable value to the pharmaceutical science. *M. krabiensis* stains in Group I and *M. marinus* strains in Groups II were distirbuted in the soils collected from Krabi, and Prajuabkirikhun whereas *M. chaiyaphumensis* strains in Group III were distributed in the soils collected from Chaiyaphume and Rattchaburi.

In addition, further studies can be carried out on the secondary metabolites produced by *Micromonospora* and *Kitasatospora*. Until now, antibiotics isolated from these two genus are still very few, this may be because the species in these genus are not so well studied. Therefore, there are possibilities that new antimicrobial substances from these new genus may be discovered in the future.

For the antimicrobial activity screening of the EtOAc extracts of the fermentation broth of twelve strains, PNK1-3 was selected for secondary metabolite production due to its crude extract showing significant antimicrobial activity against *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *M. luteus* ATCC 9341, and *C. albicans* ATCC 10231. The EtOAc extract yielded geldanamycin as a major compound. It exhibited significant cytotoxicity against human epidermoid carcinoma cell line of the nasopharynx (KB) and breast cancer cell line (BC) with

IC_{50} 0.15 and 0.01 μg/ml, respectively. It also possessed potent antimalarial activity against *Plasmodium falciparum* (K1, multidrug resistant strain) and antituberculosis at IC_{50} = 0.40 and 6.5 μg/ml. PNK1-3 possessed an antimicrobial property, which can be of remarkable value to the pharmaceutical science.

For exploration the constituents of a yellow mutant of the red yeast rice of *M. kaoliang* KB20M10.2, a crude CH_2Cl_2 extract of *M. kaoliang* KB20M10.2 grown on rice (1 kg) yielded two new yellow pigments, monascusone A (Ang01) (6,7-dihydroxy-3-(2-hydroxy-propyl)-7-methyl-1,5,6,7-tetrahydro-isochromen-8-one) and monascusone B (Ang02), together with two known compounds, monascin (Ang03) and FK17-P2b2 (Ang04). This is the first report that FK17-P2b2 was found in *M. kaoliang*. Monascusone A, the major metabolite of *M. kaoliang* KB20M10.2, was inactive against the malarial parasite (*Plasmodium falciparum*), *Mycobacterium tuberculosis* H37Ra, and *C. albicans*. Compound 1 showed no cytotoxicity against BC (breast cancer) and KB (human epidermoid carcinoma of cavity) cell lines. Unfortunately, due to the limited amount of materials, the minor metabolites, compounds 2, 3, and 4, were not tested for their biological activities. Yellow pigments, monascusone A and monascusone B were also discovered for the first time from this study. These pigments, exhibited no toxicity which is possible to be used as food or cosmetic industry. Moreover, they also have the ability to absorb UV lights. Thus, they have strong potentials of becoming useful materials in the cosmetic industry. Extracts of this red yeast rice, *M. kaoliang* KB20M10.2, may also have other minor metabolites, which are interesting to pursue the identification of these minor compounds, and HPLC-MS may be useful for this investigation.

In this study, one known species of *Streptomyces* that produced geldanamycin and three novel species of *Micromonospora* strains were isolated from Thai soils. Two new compounds and two known compounds were isolated from *M. kaoliang* KB20M10.2. This evidence showed that the microorganisms are of great interest for the investigation of new species and their secondary metabolites.