CHAPTER V

CONCLUSION

One hundred and twenty seven strains of actinomycetes were isolated from soil samples collected from Chiangrai, Nan, Phatthalung, Satun, Songkhla, Chaiyaphum, and Trat provinces, Thailand. On primary screening, most of these strains showed the antimicrobial activities against *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633 and *Micrococcus luteus* ATCC 9341, while few strains showed activities against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231. On the basis of morphological, cultural, physiological, biochemical and chemotaxonomic characteristics including phylogenetic analysis of 16S rDNA sequencing, the selected strains, S1-2 and S3-1 from soil in Chiangrai, SB12-1 and S33-3 from soil in Nan, S38-2 from soil in Phatthalung, S49-1 from soil in Songkhla, S55-4 from soil in Chaiyaphum, S71-1, S72-10, S75-3, S75-5, and S76-1 from soil in Trat were identified as *Streptomyces* (Group I). Five strains, SB7-3, KC19-1, KC20-1 from soil in Nan, S39-7 from soil in Phatthalung, and K57-1 from soil in Chaiyaphum were identified as *Amycolatopsis* (Group II). Strain SB3-2 from soil in Nan was identified as *Kitasatospora* (Group III). Spore chain morphology of selected strains were rectiflexibiles or spiral. Spores are non-motile. Colonies on agar media were powdery, granular, or velvety. Strain S1-2, S3-1, SB12-1, S33-3, S38-2, S49-1, S55-4, S71-1, S72-10, S75-3, S75-5 and S76-1 showed the same pattern of chemotaxonomic which were similar to the member of the genus *Streptomyces*. They contained LL-DAP in the cell wall peptidoglycan. Menaquinones, MK-9 (H_6) and MK-9 (H_8) were predominant. Their DNA G+C content ranged from 69.0-75.4 mol%. Strains SB7-3, S39-7, KC19-1, KC20-1, and K57-1 contained *meso*-DAP in the cell wall. The predominant menaquinone was MK-9 (H_4) as in the genus *Amycolatopsis*, which could be the differentiated them from *Streptomyces* and *Kitasatospora* strains. Their DNA G+C content ranged from 66.5-73.4 mol%. Strain SB3-2 showed the characteristics of the genus *Kitasatospora*. This strain had the same LL and *meso*-DAP in the cell wall. The predominant menaquinones pattern was the same as *Streptomyces*. DNA G+C content was 76.1 mol%. The ratio of *meso*-DAP to LL-DAP was useful to distinguish *Streptomyces, Amycolatopsis* and *Kitasatospora* strains.

Phylogenetic analysis of the almost complete 16S rDNA sequences and percentage of 16S rDNA sequence similarity revealed that strains S72-10 and S76-1 should be identified as

S. termitum (99.6 and 99.8% 16S rDNA similarity, respectively), strain S49-1 as *S. aureoversilis* (99.4% 16S rDNA similarity), strains S1-2 and S75-5 as *S. hygroscopicus* (99.8% 16S rDNA similarity), strain S38-2 as *S. aureofaciens* (99.4% 16S rDNA similarity), and strain S33-3 as *S. xanthocidicus* (99.8% 16S rDNA similarity). Strain S55-4 should be identified as *S. roseocinereus* (99.9% 16S rDNA similarity), strain S71-1 as *S. mycarofaciens* (99.4% 16S rDNA similarity), strain S75-3 as *S. albospinus* (99.4% 16S rDNA similarity), and strains S3-1 and SB12-1 as *S. spectabilis* (99.6 and 99.7% 16S rDNA similarity, respectively). Strain S39-7 was closely related to *A. albidoflavus* (99.2% 16S rDNA similarity). Strain SB7-3 was closely related to *A. keratinophila* (99.3% 16S rDNA similarity). Strains KC19-1, KC20-1 and, K57-1 were closely related to *A. kentuckyensis* (99.3, 98.1 and 99.2% 16S rDNA similarity, respectively). On the basis of phenotypic, chemotaxonomic characterictics, 16S rDNA sequences and DNA relatedness data, strains SB7-3 and S39-7 were recognized as a novel species in the genus *Amycolatopsis* which could produce antimicrobial substances that may be value to the pharmaceutical sciences. Strain SB3-2 showed 16S rDNA sequence similarity the range 98.9% with *Kitasatospora putterlickiae.* The further study on DNA-DNA relatedness will reveal the taxonomic position to be a new species of strain SB3-2. Strain SB3-2 was sensitive to low concentrations of novobiocin and hence cannot be expected to grow on the novobiocin (50 μg/ml) containing agar medium used by Tajima *et al.*, (2001).

For the antimicrobial activity screening of ethyl acetate extract of the fermentation broth of eighteen strains, strain S3-1 identified as *S. spectabilis* was selected for antimicrobial production due to its crude extract showing good antimicrobial activity. The ethyl acetate extract was tested by bioautographic method and found the active spot was shown at R_f value 0.8 (Silica gel TLC, solvent system 15% MeOH in CH_2Cl_2). This extract was isolated by chromatographic method and was shown to be active against *S. aureus* ATCC 6538, methicillin resistant *S. aureus* 266, 269, 643, *B. subtilis* ATCC 6633, *M. luteus* ATCC 9341 and *Ps. aeruginosa* ATCC 27853.

This study showed that *Streptomyces* strains were known species but *Amycolatopsis* and *Kitasatospora* strains seem to be novel species. It is interesting that the further studies on the fermentation, extraction, purification, and structure elucidation of antibiotics produced by *Amycolatopsis* and *Kitasatospora* strains should be carried out since the studies on antibiotics from these two genera are still limited in Thailand. Therefore, there are high possibilities to find the new antimicrobial substances from those strains in both genera in the near future.