CHAPTER IV

DISCUSSIONS

Streptomyces species show a variety in shape, arrangement of aerial mycelia and spore-bearing structures. The conidia and aerial mycelia are often pigmented with characteristic colors, which are fundamental features for taxonomy of the *Streptomyces* (Brock 1974). Colonies of the strains SMC 48, SMC 59 and SMC 78 shows different colors. Strain SMC 48 has white colonies and white spores, strain SMC 59 shows yellow colonie with white spores around the colony, and strain SMC 78 has pink colonies with red pigment diffused in agar, and pink spores.

One hundred strains of *Streptomyces* genomic DNA samples screened by dot blot hybridization using *picK* gene as a probe showed that twenty positively genomic DNA samples possess putative macrolide P-450. The negative -hybridization results of genomic DNA samples may not indicate that they have no macrolide P-450 gene. However, our gene probe might not complementary to other divergent macrolide P-450s.

We used degenerated primers for the PCR based on the conserved region of the known sequences of polyketide P-450 hydroxylases from *Streptomyces* to amplify an approximately 800 bp of the P-450 gene fragment. The PCR products were anticipated to have the sequence regions, which represent an oxygen-binding and heme-binding motifs near 5' and 3' ends respectively. The thirteen genomic DNA samples of local *Streptomyces* exhibited the PCR products of about 800 bp.

The PCR products of the samples (SMC 48, SMC 59 and SMC 78) were cloned for DNA sequencing and showed that their DNA and translated amino acid sequences were highly

similar to those of polyketide P-450s from *Streptomyces* origins. Amino acid sequence of SMC 48 exhibited the highest similarity to those of CYP7 from *S. avermitilis* that was predicted to be a polyketide P-450 (Ikeda *et al* 1999). Other amino acid sequence of SMC 59 were matched to the amino acid sequences of cytochrome P-450 from *S. fradiae* (Bate *et al* 1995). Amino acid sequence of SMC 78 were also matched to the amino acid sequences of cytochrome P-450 from P450-like hydroxylase from *S. rochei* (Suwa *et al* 2000). Our sequences isolated from *Streptomyces* in Thailand were in a group of the polyketide P-450 since they had the significant motifs (oxygen-binding and heme-binding) of the P-450 superfamily and showed high similarity to P-450s involved in polyketide biosynthesis. Deduced amino acid sequence of SMC48, SMC 59 and SMC 78 have the signature FXXGXXXCXG of heme-binding domain. The heme-binding domain of SMC 48 was FGYGVHLCIG, and both of SMC 59 and SMC 78 were FGIGVHHCIG. As a result, it should be mentioned that they were classified into a group of polyketide cytochrome P-450 enzymes involved in the polyketide biosynthesis.

When the secondary structures of the SMC 48, SMC 59 and SMC 78 were predicted by the nnpredict algorithm with the accuracy over 65% and were compared to that of EryF (P-450 hydroxylase) from *S. erythraea*, they revealed nine α -helices (D- L) and five β-sheets (β2-β6). The nucleotide and amino acid sequences of SMC 48, SMC 59 and SMC 78 showed genetic and functional relationship to those of cytochrome P-450 of streptomycetes, which are involved in hydroxylation of secondary metabolites as demonstrated in phylogenetic tree in Figure 36 and 37. The nucleotide and amino acid sequence of SMC 48 had close relationship to P-450 from *S. avermitilis* (AP005027), *S. griseolus* (M32239), *S. hygroscopicus* (AY179507), *S. griseus* (X63601), *S. acidiscabies* (AF393159), *S. clavuligerus* (AF200819), *S. parvulus* (AJ580915), *Streptomyces sp.* HK803 (AY354515t4), *S. carbophilus* (D30815) and *S. griseolus* (M32238). Cytochrome P-450 (*gdmP*) (AY179507) catalyzes hydroxylation in geldanamycin

biosynthesis. Cytochrome P-450 (*borI*) (AJ580915) plays a role in borrelidin biosynthesis. Cytochrome P-450 (*plmT4*) (AY354515t4) plays a role in hydroxylation in phoslactomycin B biosynthesis.

Nucleotide and amino acid sequences of SMC 59 and SMC 78 had close relationship to P-450 from *S .fradiae* (AF145049), *S. carzinostaticus* (AY1174391), *S. coelicolor* A3(2) (AL939114), *S. venezuelae* (AF087022), *S. rochei* (AB088224), *S. erythraea* (M54983F), *S. collinus* (AF293355), *S. hygroscopicus* (AF521895), *S. noursei* (AF263912), *S. nodosus* (AF357202) and *S. natalensis* (AJ278573D). Cytochrome P-450 (AY1174391) which involves in hydroxylation of neocarzinostatin biosynthesis. Cytochrome P-450 (*picK*) (AF087022) which plays role in hydroxylation in picromycin biosynthesis. Cytochrome P-450 (*eryF*) (M54983F) catalyzes hydroxylation of 6DEB in erythromycin biosynthesis. Cytochrome P-450 (*nysL*) (AF263912) involves in hydroxylation of nystatin biosynthesis. Cytochrome P-450 (*amphL*) (AF357202) which play a role in hydroxylation in amphotericin biosynthesis. Cytochrome P-450 (*pimD*) (AL278573D) involves in epoxidation in pimaricin biosynthesis.

The sizes of putative macrolide P-450 sequences of SMC 48, SMC 59 and SMC 78 were approximately 800 base pairs, but most polyketide P-450s have sequence sizes in the range of 1200-1300 bp. The sequence alignment showed that our sequences need about 200-300 bp of 5' terminus and 200 bp of 3' end because the sequence at 5' terminus is involved in the B' helix, which is a part of substrate-binding pocket and often shows sequence variation. In the future, the full-length of the predicted P-450 sequences will be unclosed by chromosome walking and be overexpressed for gene and protein characterization. These three *Streptomyces* species (SMC 48, SMC 59 and SMC 78) will be taxonomized by biochemical, microbiological and genetic methods.