論文の内容の要旨

応用生命工学　専攻
平成 25 年度博士課程　入学
氏名　インタン ティムル マイシャラ
指導教員名　西山　真

論文題目

Biochemical Studies on ABBA Prenyltransferases from *Streptomyces*
（放線菌由来 ABBA プレニルトランスフェラーゼに関する生化学的研究）

Prenylated natural products are produced by terrestrial and marine organisms, many of which exhibit important biological and pharmacological activities. Attachment of prenyl group for generating prenylated metabolites is catalyzed by prenyltransferase (PT), and is considered to improve the pharmacological properties of natural products. ABBA PT superfamily is a relatively new family of enzymes, as a subgroup of aromatic substrate PTs that hold five α-β-β-α secondary structure repeating elements, termed as PT barrel fold.

To date, a number of ABBA PTs have been identified and characterized. Many reports have shown the promiscuity of ABBA PT members capable of accepting various indole- or phenol-containing substrates. As ABBA PT enzymes are soluble protein with high promiscuity to accept various substrates, they are very potential to be utilized as biocatalysts to produce prenylated derivatives by chemoenzymatic synthesis. Currently, there are still limited reports concerning utilization of ABBA bacterial PTs toward indole compounds. Furthermore, compared to other classes of ABBA PTs, bacterial indole ABBA PTs are less numbered. The work presented in this study includes bacterial PT enzymes belonging to the ABBA PT superfamily and their potential as biocatalyst in chemoenzymatic synthesis. Additionally, the availability of bacteria genome sequences has enabled to identify numerous potential

genes responsible for secondary metabolite biosynthesis. By genome mining, two putative PTs were identified in *Streptomyces* strains. Biochemical studies of these two novel PTs demonstrate that they are phenol-*O*-prenyltransferase, which are categorized into a new subgroup in ABBA bacterial PTs.

Chapter 1 SCO7467: Promiscuous tryptophan dimethylallyltransferase from *Streptomyces coelicolor* A3(2) as biocatalyst for chemoenzymatic synthesis of prenylated indoles

This chapter describes the utilization of a bacterial indole PT, SCO7467, 5-dimethylallyltryptophan synthase (5-DMATS) from *Streptomyces coelicolor* A3(2), as a biocatalyst to produce prenylated indole derivatives by chemoenzymatic synthesis. The substrate specificity of the enzyme was determined by employing various aromatic substrates in *in vitro* reactions, followed by characterization of reaction products by NMR and HRMS. SCO7467 was revealed to display wide substrate specificity. The prenylation mostly occurred at C-6 of the indole substrates tested, whereas the natural substrate L-tryptophan was prenylated at C-5. Diprenylation at C-5 and C-6 also occurred in N-containing substrate in 3-substituent. Interestingly, prenylation at C-3 followed by further structure modification were found in the *in vitro* reaction of SCO7467 with DMAPP and indole-3-acetonitrile.

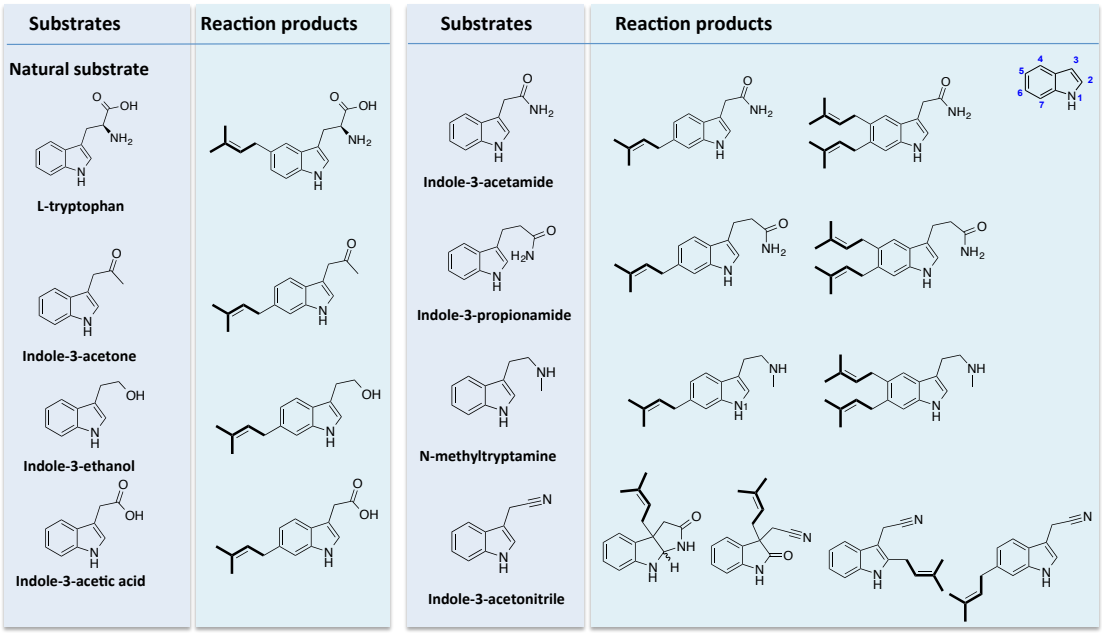

Figure 1. *In vitro* reactions catalyzed by SCO7467

Chapter 2 RI18_6032: ABBA indole prenyltransferase-tryptophanase fused protein from *Streptomyces* sp. RI18

A fusion protein of ABBA indole prenyltransferase-tryptophanase was identified in the genome of *Streptomyces* sp. RI18. The enzyme was overexpressed as N-terminal His-tagged protein, and employed in the *in vitro* reaction with L-tryptophan and DMAPP as substrates and pyridoxal phosphate (PLP) as coenzyme. Prenylation products of tryptophan and PLP-bound tryptophan were identified as reaction products, whereas the activity of tryptophanase was not detected.

Chapter 3 JL68_2352: New tyrosine-*O*-prenyltransferase of bacterial ABBA prenyltransferase from *Streptomyces versipellis* 4083-SVS6

JL68_2352 was identified in the genome of *Streptomyces versipellis* 40183-SVS6 to encode an ABBA PT homolog. However, the putative PT gene is not clustered with genes necessary for related secondary metabolism. JL68_2352 was expressed as N-terminal His-tagged protein, with estimated molecular mass of 45.5 kDa according to gel filtration analysis, indicating that it is most likely a monomer in the solution. In contrast to most bacterial ABBA PTs, JL68_2352 did not accept tryptophan as a prenyl acceptor, but accepted tyrosine to generate *O*-prenylation product of tyrosine. This result indicates that JL68_2352 should be categorized into a phenol-*O*-prenyltransferase subgroup. JL68_2352 is the first enzyme categorized into the subgroup of bacterial indole PTs in the ABBA PT superfamily. The kinetic study of JL68_2352 toward tyrosine at the saturated concentration of DMAPP revealed that the enzyme followed Michaelis-Menten kinetics. The substrate specifity of JL68_2352 toward aromatic substrates was found to be strictly specific to tyrosine.

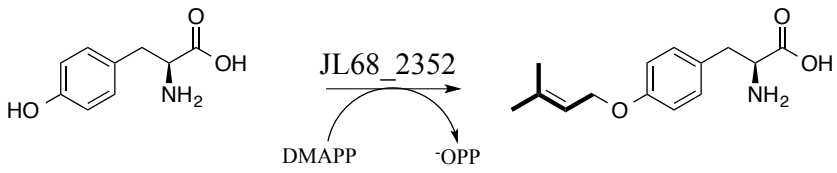

Figure 2. Reaction catalyzed by JL68_2352

Chapter 4 PTS22: New phenol-*O*-prenyltransferase of bacterial ABBA prenyltransferase from *Streptomyces* sp. SS080624GE-03

A novel ABBA PT homolog PTS22 found by genome mining was identified from *Streptomyces* sp. SS080624GE-03. To identify the metabolites derived from the activity of PTS22, heterologous expression of PTS22 was carried out using *S. lividans* TK23 as a host strain. Two compounds were identified as *O*-prenylated products of 4-hydroxybenzoic acid. The prenylation at hydroxyl group of the molecule suggested that PTS22 is a member of new subgroup of ABBA bacterial indole PTs, together with JL68_2352 from *S. versipellis* 4083-SVS6, which catalyzes *O*-prenylation of phenolic substrates. Unfortunately, protein expression by using *E. coli* expression system resulted in formation of inclusion body. Therefore, substrates specificity and further features of this novel prenyltransferase still remain elusive.

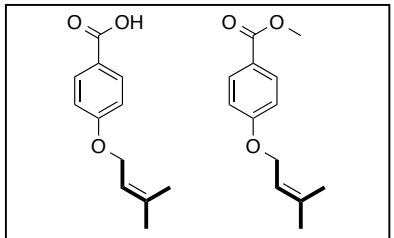

Figure 3. Isolated compounds from heterologous expression of PTS22 in *S. lividans* TK23

Conclusions

This study demonstrated the promiscuity of bacterial indole PT members as potential biocatalysts in the chemoenzymatic synthesis of prenylated indole derivatives. Since the prenylation improves the pharmacological properties of natural products, PTs could become important tools in drug discovery and drug design. This study also revealed a new class of enzymes as a subgroup of bacterial indole PTs, termed as phenol-*O*-prenyltransferases. This enzyme group does not accept indole compound as a substrate and possesses gene cluster profiles that notably differ from those of other known bacterial indole PTs.

The number of ABBA PT superfamily has been expanding since the discovery about 10 years ago. The availability of genome sequences increasing tremendously in recent years allows the identification of more potential genes coding for this enzyme family proteins by genome mining. The results of this study provide insight into the divergence of prenylation reactions catalyzed by this enzyme family, particularly in bacteria indole PTs group.