# Isolation and structure determination of bioactive peptides from actinobacteria based on genome mining

（課程博士・様式７）（Doctoral qualification by coursework,Form 7）

# 学 位 論 文 要 旨

## Abstract of Doctoral Thesis

専　　攻：バイオサイエンス　　氏　名：イサラ　カウィワン
Course：Bioscience　　Name：Issara Kaweewan

論文題目：ゲノムマイニングに基づく放線菌由来生理活性ペプチドの単離と構造決定
Title of Thesis : Isolation and structure determination of bioactive peptides from actinobacteria based on genome mining

Abstract：

Actinobacteria are Gram-positive bacteria with high guanine and cytosine content in DNA found in terrestrial environments, mainly in soil and aquatic environments including freshwater and marine. They have been recognized as producers of wide variety of bioactive compounds with diverse biological functions, such as antibacterials, antivirals, antitumors, immunosuppressives and enzyme inhibitors. More than half of commercial antibiotics are produced from actinobacteria especially *Streptomyces* species. Recently, genome mining approach has become a useful tool for discovery of new bioactive peptides from bacteria. This approach is a genetic screening strategy to predict potential biosynthetic gene clusters (BGCs) of peptide natural products in bacterial whole genome sequences accumulated in database. So far, several bioactive peptides have been discovered based on genome mining.

Bioactive peptides of bacterial origin are classified based on biosynthetic pathways into two major groups, including ribosomally synthesized and post-translationally modified peptides (RiPPs) and peptides biosynthesized by nonribosomal peptide synthetases (NRPSs). RiPPs are initially synthesized as a precursor peptide comprising of leader peptide and core peptide regions. The core peptide is further modified by modification enzymes to afford a final product. Nonribosomal peptides (NRPs) are synthesized by nonribosomal peptide synthetases (NRPSs). The module in an NRPS is responsible for the incorporation of one specific amino acid into the final product like building blocks. NRP biosynthesis occurs in several steps, including the processes of chain initiation, elongation and termination on the assembly line as well as post translational modification by tailoring enzymes. According to the biosynthetic pathways, RiPPs and NRPs are suitable targets for genome mining.

In this study, three new bioactive peptides including RiPPs and NRP were discovered from *Streptomyces* based on genome mining. The peptides were isolated from extract of bacterial cells by HPLC separation. Chemical structures of bioactive peptides were determined by combination of ESI-MS and NMR analyses. Modified Marfey’s analyses were conducted to elucidate absolute configurations of peptides. Biological activities of peptides such as antibacterial, anti-HIV, and cytotoxicity were clarified and biosynthetic gene clusters (BGCs) of peptides were proposed in bacterial genome sequences. The study includes three chapters. The details of study were described as following;

In chapter I, a new lasso peptide structural gene was found in the genome sequence of *Streptomyces specialis* by genome mining. The peptide named specialicin was isolated from the MeOH extract of *S. specialis* JCM 16611$^{T}$. Specialicin was determined as a new lasso peptide with a length of 21 amino acids, containing isopeptide bond and two disulfide bonds by ESI-MS and NMR analyses. Three dimensional structure of specialicin indicated that specialicin possessed similar conformational structure with a known peptide siamycin I (MS-271). Interestingly, stereochemistry of Trp at C-terminus of specialicin was determined to be D-configuration. The BGC for specialicin, *spe* cluster, was proposed in the genome sequence of *S. specialis*. Genes within *spe* cluster were annotated based on similarity to *msl* cluster, responsible for MS-271 biosynthesis. Specialicin showed moderate anti-HIV activity against HIV-1 NL4-3 and antibacterial activity against Gram-positive bacteria, *Micrococcus luteus*.

In chapter II, a new precursor peptide coding gene was found in the genome sequence of *Streptomyces curacoi* by genome mining. Since *Streptomyces* harboring RNA polymerase β gene (*rpoB*) mutation which confers rifampicin resistance can produce high quantities of bioactive peptides, rifampicin-resistant (rif$^{r}$) mutants of *S. curacoi* were constructed by spontaneous mutation. The peptide named curacozole was isolated from the MeOH extract of rif$^{r}$ mutants of *S. curacoi* NBRC 12761$^{T}$. Using ESI-MS and NMR analyses, chemical structure of curacozole was determined to be a macrocyclic peptide containing two isoleucine, two thiazole and three oxazole, which similar to a known peptide, YM-216391. Curacozole was proposed to be biosynthesized from precursor peptide by several enzymatic modifications. The BGC for curacozole was identified in the genome sequence of *S. curacoi* based on similarity to *ym* gene cluster, responsible for YM-216391 biosynthesis. Curacozole exhibited potent cytotoxicity against HCT116 and HOS cancer cells and induced HCT116 cell apoptosis.

In chapter III, chemical investigation on extract of actinobacteria indicated presence of a new antibacterial peptide, named pentaminomycin C, in the extract of *Streptomyces cacaoi* subsp. *cacaoi* NBRC 12748$^{T}$. The peptide pentaminomycin C, along with known peptide BE-18257A, were isolated from the MeOH extract of *S. cacaoi*. Using ESI-MS and NMR analyses, chemical structure of pentaminomycin C was determined to be a cyclic pentapeptide containing unusual amino acid, 5-OHArg, in the molecule. The BGC including two NRPS genes for pentaminomycin C and BE-18257A was identified in the genome sequence of *S. cacaoi* subsp. *cacaoi* NBRC 12748$^{T}$. Pentaminomycin C showed antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus luteus*, but BE-18257A did not showed any antibacterial activity against test bacteria.

Above all, three new bioactive peptides were discovered based on genome mining. This study contributed not only to discovery of new drug seed compounds, but also to accumulation of information on biosynthesis of peptides.