Diketopiperazines (DKPs) constitute a family of secondary metabolites which are mainly synthesized by microorganisms. In recent years, a lot of interest has been shown in DKPs as these molecules exhibit a wide variety of biological activities. For instance, albonoursin shows antibacterial and antitumor activities\(^1\), cyclo(L-Phe-trans-4-OH-L-Pro) acts as an antifungal compound\(^2\) and gliotoxin exhibits antiviral and immunosuppressive properties\(^3\). The DKP scaffold is commonly known to be synthesized by nonribosomal peptide synthases (NRPSs)\(^4\). NRPSs are large multifunctional enzymes comprising of various modules, wherein, each module is responsible for incorporating one amino acid in the growing polypeptide. Various domains are present in each module such as an adenylation domain for activating an amino acid as an amimoacyl AMP moiety, a peptidyl carrier protein domain where the activated amino acids are transferred, a condensation domain for peptide bond formation between aminoacyl substrates and a transesterification domain for the release of the final peptide.

In the last decade, a new class of enzymes has evolved which hijack activated amino acids in the form of aa-tRNAs and use them as substrates for the synthesis of various cyclodipeptides. These enzymes are known as cyclodipeptide synthases or more commonly as CDPSs. AlbC was the first member of this family to be discovered from *Streptomyces noursei* in 2002\(^5\).

Till date only three CDPS-dependent pathways have been fully characterized, namely,

1) Albonoursin biosynthesis in *Streptomyces noursei*.
2) Mycocyclosin biosynthesis in *Mycobacterium tuberculosis*.
3) Pulcherrimin biosynthesis in *Bacillus subtilis*. 
Biosynthesis of albonoursin

AlbC is a 239 amino acids residue monomeric protein. It uses Phe- tRNA\textsubscript{Phe} and Leu- tRNA\textsubscript{Leu} as substrates for the synthesis of the cyclo(L-Phe-L-Leu) (cFL). The AlbC structure\textsuperscript{6} consists of a Rossmann fold domain and is highly similar to the catalytic domain of class-I aminoacyl-tRNA synthetases, particularly, class-Ic TyrRSs from *Methanococcus jannaschii* and TrpRSs from *Entamoeba histolytica*. The amino acid residues in the active site of AlbC found to be essential for its enzymatic activity include Y178, E182, S37 and G35. The serine-37 residue has been found to act as a nucleophile leading to the formation of a covalent enzyme-substrate intermediate. The hydroxyl group of Y178 and the carboxylate group of E182 form hydrogen bonds with the amino group of the substrate while the G35 residue is important for the accommodation of the CDPS substrate. A ping pong mechanism has been proposed, wherein, a covalent phenylalanyl-enzyme intermediate\textsuperscript{6} is formed as shown in figure 3.

![Figure 3: Mechanistic proposal for the formation of covalent substrate-enzyme intermediate](image)

After the formation of this covalent intermediate with the first aa-tRNA substrate, the aminoacyl-enzyme can then react with the aminoacyl moiety of the second aa-tRNA leading to the formation of a dipeptidyl-enzyme or dipeptidyl-tRNA intermediate which can finally undergo intramolecular cyclization to form the cyclopeptide as the final product.

Cyclodipeptide (cFL) synthesized by AlbC serves as substrate for cyclic dipeptide oxidase (CDO)\textsuperscript{7} which converts it into albonoursin cyclo(α,β-dehydro Phe-α,β-dehydro Leu) (cΔFΔL). CDO is a novel, amino acyl α,β-dehydrogenase isolated from *Streptomyces noursei*. It catalyzes
the formation of albonoursin in a two step sequential manner using covalently bound flavin as a cofactor.

**Biosynthesis of mycocyclosin**

![Biosynthetic pathway of mycocyclosin](image)

Rv2275 is a cyclodityrosine synthetase which catalyzes formation of cyclo(L-Tyr-L-Tyr) (cYY) in *Mycobacterium tuberculosis* using Tyr-tRNA\textsuperscript{Tyr} as substrates. It is a dimeric protein consisting of 289 amino acids residue and shows 26% sequence identity with AlbC. The catalytic pocket residues in Rv2275 superimpose fairly well upon those of AlbC.

Cyclodipeptide (cYY) is further converted into mycocyclosin by a cytochrome P450 enzyme, CYP121\textsuperscript{9} in *Mycobacterium tuberculosis*. CYP121 is known to be essential for the viability of *Mycobacterium tuberculosis* and is inhibited by azole antifungal drugs such as clotrimazole, econazole, fluconazole and ketoconazole. CYP121 uses an electron transport chain comprising of NADPH, ferredoxin reductase and ferredoxin for its activity. It catalyzes an intramolecular C-C bond formation between two tyrosyl carbon atoms of cYY via a biradical mechanism. The identification of the above transformation catalyzed by CYP121 may turn out to be very useful in the designing of antimycobacterial drugs. Substrate analogues can be designed which can serve as potent CYP121 inhibitors.

**Biosynthesis of pulcherrimin**

![Biosynthetic pathway of Pulcherrimin](image)

YvmC is another CDPS enzyme found in *Bacillus subtilis* which catalyzes the formation of cyclodileucine (cLL) using two Leu-tRNA\textsuperscript{Leu} molecules as its substrate.\textsuperscript{10} CYP134A1\textsuperscript{11} which is a cytochrome P450 oxidizes cLL into pulcherriminic acid which further chelates with Fe\textsuperscript{3+} ion leading to the formation of a red pigment pulcherrimin.

Thus, cyclodipeptide synthases (CDPSs) represent a novel class of enzymes that catalyze DKP scaffold biosynthesis using activated amino acids as substrates. Characterization of new CDPSs will lead to new possibilities for pathway engineering which may open up opportunities to generate new products with interesting biological properties.
References