Full Abstract

**Identification and Engineering of Nonribosomal Peptide Biosynthetic Systems.**

Nonribosomal peptides represent an important family of natural products, including antimicrobials, anticancer agents, immunosuppressants, siderophores, herbicides, antifungals, insecticides, and anthelmintics. They are biosynthesized by giant modular enzymes called nonribosomal peptide synthetases (NRPSs). In my doctoral dissertation research, I identified a new indigoidine biosynthetic gene cluster and reconstituted this indigoidine biosynthetic pathway in two different heterologous hosts, *S. coelicolor* CH999 and *E. coli* BAP1. The blue pigment indigoidine is synthesized by condensation of two units of L-glutamine by an activated NRPS. This pigment can be easily extracted and quantified. Under the optimal fermentation conditions, the yield of indigoidine reached 2.78 g/l in *E. coli* BAP1. To further improve the production of indigoidine, a metabolic engineering technique was used to directly enhance the *in situ* supply of this precursor. Engineering of a glutamine synthetase GS into the *E. coli* host followed by the addition of the ammonium salt (NH$_4$)$_2$HPO$_4$ at 2.5 mM led to the production of indigoidine at 7.08 g/l.

Cyclooligomer depsipeptides (CODs) are a particular group of nonribosomal peptides often found in fungi as mycotoxins, such as beauvericins, bassianolide and ennatinins. We examined the heterologous reconstitution of fungal CODSs in *S. cerevisiae* and production of the corresponding anticancer CODs. The activities of two fungal NRPSs, BbBEAS and BbBSLS, were reconstituted efficiently in *S. cerevisiae* BJ5464-NpgA. The titers of the anticancer natural products, beauvericins and bassianolide, are comparable to those in the native producer. I also dissected the two modular NRPSs into modules and reconstituted the biosynthetic pathways by co-expressing the fragments in *S. cerevisiae*. The linker between M1 and M2 plays a pivotal role in the reconstitution of the dissected NRPSs. We also reconstruct the total biosynthesis of beauvericin *in vitro* by reacting C$_2$ and C$_3$ with two SNAC-linked precursors and present a domain swapping approach to reprogramming these enzymes for peptides with altered lengths. These findings highlight the difference between bacterial and fungal NRPS mechanisms and provide a framework for the enzymatic synthesis of non-natural nonribosomal peptides.