Investigation and Engineering of Polyketide Biosynthetic Pathways | Biological Engineering

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Lei Sun

Dissertation Defense
Candidate for PhD in Biological Engineering

Advisor - Dr. Jixun Zhan

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Full abstract

Abstract

Polyketides are a large family of natural products found in bacteria, fungi and plants, which include many clinically important drugs such as tetracycline, chromomycin, spirolaxine, endocrocin and emodin. They are synthesized from acyl- or malonyl-CoA precursors by polyketide synthases (PKSs). Three types of PKSs are known to date. Type I PKSs are multifunctional enzymes that are organized into modules, each of which harbors a set of distinct, non-iteratively acting domains responsible for the catalysis of one cycle of polyketide chain elongation. Type II PKSs are multienzyme complexes that carry a single set of iteratively acting enzymes. Type III PKSs, also known as chalcone synthase-like PKSs, are homodimeric enzymes that essentially are iteratively acting condensing enzymes.

In the first part of my dissertation research, I identified a type I nonreducing PKS (NR-PKS) EmoA and a discrete thioesterase EmoB from fungi Shiraia sp. strain Sfp14 which produces endocrocin and emodin, and heterologously expressed these enzymes in the industrial strains Saccharomyces cerevisiae and Pichia pastoris. In order to improve the production of target compounds endocrocin and emodin, we co-expressed a more efficient thioesterase ACTE1 with EmoA and introduced an acetyl-CoA carboxylase ACC1 into the biosynthetic pathway, which provides more malonyl-CoA for the polyketide biosynthesis. A decarboxylase TpcK was also introduced to further increase the production of emodin by converting endocrocin. The second part was to characterize a silent type II PKS gene cluster involved in the biosynthetic pathway of chromomycins in Streptomyces resescleroticus and optimize the production of chromomycins by engineering two regulatory genes to construct an efficient producing strain. The third part of this research was to identify a type III PKS involved in the biosynthesis of spirolaxine (anti-Helicobacter pylori) in a fungal strain Sporotrichum laxum. Heterologous expression of this PKS gene in Escherichia coli produced alkylresorcinols which serve as the backbone to synthesize spirolaxine. In the fourth part of my research, we manipulated the fermentation broth of E. coli harboring a type III PKS StTS by supplying different nutrients including glucose and sodium pyruvate at different concentrations, from which six novel flaviolin derivatives were produced. It provides a new approach to biosynthesizing new molecules in the widely used heterologous host E. coli.

One goal of my dissertation research was to investigate and engineer the biosynthetic pathway of pharmaceutically valuable compounds, such as chromomycin, spirolaxine and emodin, so that we can produce the target compounds more efficiently for potential applications in industry. The other goal was to establish E. coli as a platform, for heterologous expression of PKSs and engineering of particular biosynthetic pathways to generate chemical diversity in natural products, such as novel flaviolin derivatives and alkylresorcinols.