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Review Article

Heterologous Biosynthesis of Polyketides in *Escherichia coli*

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Abstract

Polyketides represent a class of secondary metabolites and a structurally diverse family of natural products with biological activities and pharmacological properties. They exist widely in animals, plants, fungi and bacteria. Polyketides are produced by Polyketide Synthases (PKSs) and are often modified into complex bioactive compounds. PKSs are generally divided into three classes: type I, II, and III PKSs. All of those PKSs produce highly diverse polyketide chains by sequential condensation of the starter units with extender units. Due to their great diversity in structure and mechanism, tremendous advances have been made in biosynthesizing polyketides in various microorganisms. In this mini-review, we will summarize the most recent progress in metabolically engineering polyketide biosynthesis in *E. coli* and discuss the opportunities for generating novel polyketide compounds.

Keywords: Combinatorial Biosynthesis; Metabolic Engineering; Polyketides; Polyketide Synthases; Synthetic Biology.

Introduction

Polyketides are a type of structurally diverse and biologically active secondary metabolites derived from natural sources such as animals, plants, fungi and bacteria. Due to their antibiotic, antitumor, anti-inflammatory, antifungal and antiparasitic properties, these compounds are widely used as clinical medicines for treatment of various acute and chronic diseases [1]. Basically, polyketides are synthesized from condensation of thioesters by Polyketide Synthases (PKSs). Based on their catalytic mechanism, PKSs can be classified into three types, type I, II, and III PKSs [2]. Type I PKSs have large and multiple domains consisting of Iterative Type I Pkss (iPKSs) and Modular Type I Pkss (mPKSs). IPKSs, similar to fatty acid synthase, are a single enzyme with a series of domains catalyzing elongation of carbon chain and regeneration of functional group. MPKSs, like non-ribosomal peptide synthetases, are composed of a sequence of separate modules, and contain sets of domains and/or multiple catalytic domains for each elongation cycle non-repeatedly [1,2]. Thus, type II PKSs are aggregates of mono-functional proteins, repeatedly use same domains to catalyze the condensation reaction and chain elongation, which have the similar catalytic mechanism but different enzyme structure compared with iPKSs [3,4]. Type III PKSs are different from both of above two types, which contain small and simple homodimers. Each monomer catalyzes the starter unit activation, extender unit condensation cycles and chain termination iteratively to generate polyketide products [5,6].

PKSs have been found widely present in animals, plants, fungi and bacteria. Initial efforts were mostly focused on engineering of polyketide biosynthesis pathways in *Streptomycetes* and *Actinomycetes* species [1,5,7]. Although these microorganisms are primary hosts for the production of polyketides, they usually suffer from various disadvantages including difficulty in cultivation, slow growth, lack of genetic manipulation tools, and poorly understood genetic background. *Pseudomonas putida* and *Saccharomyces cerevisiae* are two candidates for production of polyketides, but these two microorganisms are difficult for high throughput genetic manipulation because of intricate DNA operational tools and lack of large gene clusters transformation approach. In contrast, *Escherichia coli* as a model microorganism, grows fast and has well-understood genetic background and abundant DNA manipulation tools, which renders it a very promising host for polyketide biosynthesis [8-11] (Table 1). Recent metabolic engineering and synthetic biology efforts have enabled efficient production of various high-value natural products in *E. coli*, such as alkaloids, terpenoids, phenolic acids, and non-proteinaceous amino acids [12-19]. With the development of synthetic biology, more and more new technologies, such as DNA assembly techniques, stress-response promoters and CRISPR/Cas9, were applied for metabolic engineering in *E Coli.* Thus, *E. coli* is a versatile and promising micro-

bial factory for production of complex compounds. In this article, we will briefly review the recent progress in polyketide production in *E. coli* and provide prospects on generating novel polyketides via metabolic engineering and synthetic biology approaches.

Advantage	Disadvantage
Grows fast	Sensitive to phage
Utilization of cheap medium	Poor resistance of PKS
Simple culture condition	Lack of posttranslational modification
Clear genetic background	Low concentration of precursor
Various genetic tool	
Simple operational tools	
High efficient protein expression system	
Less by-product	
Low pathogenicity	
Industrial-scale production	

Table 1: The Advantage and Disadvantage of Using *E. coli* for Heterologous Expression of Polyketides.

Polyketides by Type I PKSs

Type I PKSs are huge assembly of multifunctional enzymes, consisting of a series of domains separated by short spacer regions. Their encoding genes are assembled in one gene cluster. These domains contain AcylTransferase (AT), Acyl Carrier Protein (ACP), KetoSynthase (KS), KetoReductase (KR), DeHydratase (DH), EnoylReductase (ER), MethylTransferase (MT) and Thioesterase (TE). iPKSs and mPKSs share analogical structure and catalytic mechanism [1,2]. However, iPKSs use the same domains repeatedly to extend carbon chain, while mPKSs use a sequence of separate modules: starting module, elongation module, and termination module for each elongation cycle (Figure 1) [20].

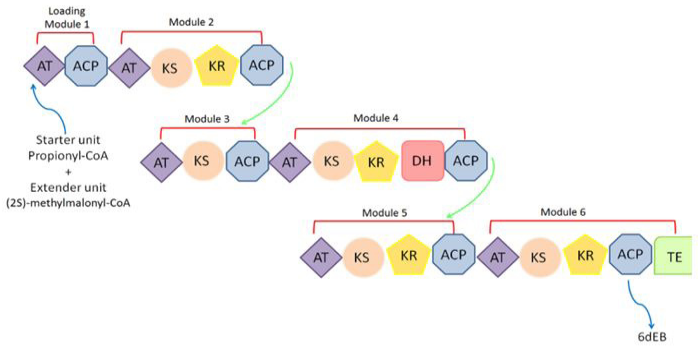

Figure 1: Biosynthesis of 6dEB by modular type I PKSs. 6-deoxyerythronolide B (6dEB) is synthesized from condensation of starter unit propionyl-CoA with extender unit (2S)-methylmalonyl-CoA. AT, acyl transferase domain; ACP, acyl carrier protein domain; KS, ketosynthase domain; KR, ketoreductase domain; DH, dehydratase domain; TE, thioesterase domain.

IPKSs are responsible for the biosynthesis of bioactive natural molecules like antiviral and antibacterial agents in plants. Thus, it was believed that iPKSs were specific to plants until iPKSs were recently(?) discovered in many kinds of bacteria [21]. However, very few studies have been reported for the expression of iPKSs in *E. coli* for polyketide biosynthesis. Recently, Kage, et al. employed λ Red-mediated cloning strategy to assemble a truncated enzyme and the first extender module (A1-ACP-KS-AT-KR-ACP) from *Ralstonia solanacearum*in *E. coli.* Via site-directed mutagenesis, the crucial KetoReductase (KR) domain for correct substrate processing was selected and successfully led to the production of 6-pentylsalicylic acid, an important medicine precursor [21]. This research provided an approach for functionally assembling fungal polyketide synthases in bacteria and substrate directed evolution. IPKSs are not only important enzymes for producing secondary metabolites but also powerful tools for generating hydrocarbons. Previously, one iPKS termed SgcE was employed to produce enediyne core in *Streptomyces globisporus* [22]. Based on that, more work has been done to further generate hydrocarbons using this iPKS. A TE domain SgcE10 was co-expressed with SgcE, which led to the accumulation of PentaDecaHeptaene (PDH) in *E. coli* [23,24]. Liu and co-researchers produced almost 140 mg/L Penta Decane (PD), derived from hydrogenation of PDH, by using synthetic promoters to finely tune the SgcE10: SgcE ratio in *E. coli* [25].

MPKSs are mainly responsible for synthesis of macrolide antibiotics like erythromycin, which is a complex compound that contains a lactone ring (6-deoxyerythronolide B, 6dEB) and two deoxysugar units (L-mycarose and D-desosamine) [1,26]. Erythromycin is usually synthesized by the soil-dwelling bacterium *Saccharopolyspora erythrae* and has been used as a medicine against Gram-positive bacterial infections for a long period [26,27]. Several years ago, Pfeifer and his colleagues firstly reported engineering *E. coli* to convert exogenous propionate into lactone ring 6dEB, a vital part of erythromycin (Figure 1). This was the first work for biosynthesis of the precursor of erythromycin in *E. coli* [26]. It is noteworthy that they further achieved *de novo* biosynthesis of erythromycin and its derivatives in *E. coli*. 10 mg/L of erythromycin A was produced by transferring 3 large polyketide synthase genes (~ 30kb) and extra 17 genes responsible for generating deoxysugar unit of erythromycin into *E. coli*, indicating that *E. coli* can be used as a chassis for production of erythromycins [27]. Since propionyl-CoA and (2S)-methylmalonyl-CoA (Figure 1) were the starter and the extender units for the biosynthesis of erythromycin, three different pathways were introduced into *E. coli* for generating propionyl-CoA from citramalate and threonine. This approach was not only effective towards generating erythromycin but also produced benzyl-erythromycin analog [28]. Recently, Pfeifer and his co-workers modified the mycarose unit by introducing 16 tailoring pathways to heterologously biosynthesize diversiform erythromycin analogs in *E. coli*. This work focused on redesign of deoxysugar pathways to expand the scope of native

mycarose moiety by using putative bioreactions. Eventually, 42 erythromycin derivatives were produced by different combinations of 16 mycarose units and 4 erythromycins (Figure 2). Besides, their antibiotic activities were tested against several well-characterized *Bacillus subtilis* strains that are resistant to erythromycin, chloramphenicol and streptomycin. The data revealed that allthese new erythromycin analogs are active against the chloramphenicol- and streptomycin-resistant *B. subtilis*strains, while only 3 of them are effective against the erythromycin-resistant *B. subtilis* strains. Those results provided guidance for further development of new antibiotic analogues with enhanced activities [29]. In order to accumulate precursors, up-regulation of deoxysugar glycosyltransferase gene by increasing this gene copy numbers and re-direction of the carbon flux of metabolic network, led to 7-fold increase of erythromycin A production in *E. coli.* This approach used different plasmids and is very efficient. However, it will increase the metabolic burden and the plasmids are prone to be eliminated. The expression plasmids, which were needed for biosynthetic pathway introduction, led to 5-fold increase of erythromycin A production.

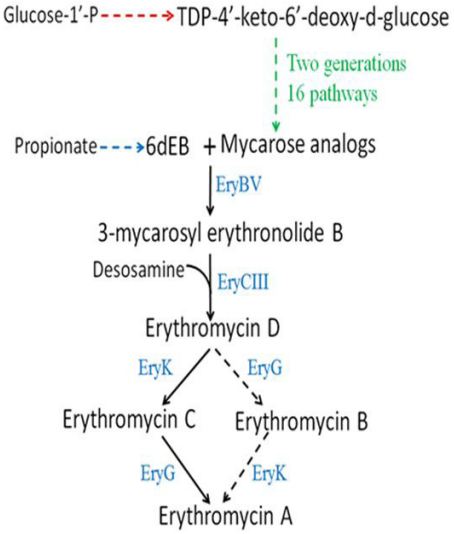

Figure 2: Engineered biosynthesis of erythromycin and its derivatives in E. coli. Red dashed arrow represents the native pathway in E. coli. Green dashed arrow indicates 16 tailoring pathways. Blue dashed arrow indicates an engineered pathway was introduced in E. coli. Black solid arrows indicate the main reaction steps. Black dashed arrows show side reaction steps. 6dEB: 6-deoxyerythronolide B; EryBV: dTDP-D-mycarose glycosyltransferase; EryCIII: 3-L-mycarosyl erythronolide B desosaminyltransferase complex; EryK: C12 hydroxylase; EryG: C3'-O-methyltransferase.

Polyketides by Type II PKSs

Different from type I PKSs, type II PKSs are aggregates of monofunctional proteins, containing a ketosynthase-chain length factor (KSα-CLF) complex. They are mainly responsible for producing aromatic polyketides by catalyzing iterative Claisen condensation using malonate as the substrate [4]. Although the catalytic mechanism of type II PKSs is clear, only a few of studies have been reported for heterologous expression of type II PKSs in *E. coli* to generate polyketides so far.

Recently, Stevens,et al. successfully engineered *E. coli* to produce up to 2 mg/L oxytetracycline by co-expressing alternative sigma factor $\sigma^{54}$with the PKS from oxytetracycline biosynthesis pathway in *S. rimosus* [30]. This work showed that the expression level of transcriptional regulators had significant effects on oxytetracycline production. Lomaiviticin, an important class of diazo-containing aromatic polyketides, gained great interests because of its antibiotic and antitumor activities. Waldman and his colleagues obtained a gene cluster responsible for the biosynthesis of lomaiviticin in *Salinispora pacifica* DPJ-0019. They successfully expressed three key lomaiviticin biosynthetic enzymes: a bifunctional enzyme of AcylTransferation and DeCarboxylation (AT/DC), LnmK encoded by *lom62* and two ACPs encoded by *lom60* and *lom63* in *E. coli* [4]. *In vitro* enzyme assay showed all three enzymes functioned well, thereby exhibiting the potential for achieving *de novo* biosynthesis of lomaiviticin in *E. coli*.

Polyketides by Type III PKSs

Different from above two type PKSs, type III PKSs are small and simple enzymes catalyzing starter unit activation, extender unit condensation cycles and chain termination in a single active site to generate a diverse of natural products such as flavonoids, acridones, pyrones and stilbenes (Figure 3)[5,6,31].

Flavonoids are well-studied plant polyketides, synthesized by Chalcone Synthase (CHS) (Figure 3). CHS catalyzes the first step of flavonoids biosynthesis by condensation of starter unit 4-coumaroyl-CoA with three acetate parts from malonyl-CoA. Naringenin chalcone is a common intermediate and is sequentially converted into flavonoid backbone by Chalcone Flavanone Isomerase (CHI). With further decoration and modification by various tailoring enzymes, thousands of flavonoids have been generated [31,32]. Recently, heterologous biosynthesis of flavonoids using type III PKSs has made tremendous progress in *E. coli*. Kim, et al. used naringenin biosynthesis pathway to produce sakuranetin (7-O-methylnaringenin) and ponciretin (4'-O-methylnaringenin) in *E. coli*. Up to 42.5 mg/L ponciretin and 40.1 mg/L sakuranetin were produced by co-expressing several genes for ponciretin and sakuranetin biosynthesis, up-regulating shikimate pathway, and disrupting isocitrate dehydrogenase to accumulate the precursor-coenzyme A [33]. This article demonstrated an efficient strategy for sakuranetin and made a possible to explore the biosynthesis of ponciretin and sakuranetin. Another study reported engineering *E. coli* by over-expressing its native UDP-glucose dehydrogenase and UDP-xylose synthase from *Arabidopsis thaliana* enabled the production of 160 mg/L quercetin 3-O-xyloside and quercetin 3-O-arabinoside, which are two kinds of plant-specific flavonoids [34]. Stahlhut and his co-workers firstly achieved the production of garbanzol and resokaempferol from tyrosine in *E. coli* via introduction of nine heterologous enzymes [35]. Over-expression of key enzymes and deletion of competing pathways are powerful and traditional metabolic engineering strategies used in heter-

ologous biosynthesis of natural products. Lim and co-researchers engineered Cyanidin 3-O-Glucoside (C3G) biosynthetic pathway by identifying transport proteins responsible for the uptake of catechin and efflux of C3G, optimizing plasmid construct configuration, augmenting the intracellular availability of UDP-glucose and improving the culture conditions, resulting in accumulation of 350 mg/L C3G, which is the highest production reported so far [36]. Jeong,et al. reported that through co-expression of cinnamate/4-coumarate: coenzyme A ligase, stilbene synthase and resveratrol O-methyltransferase which are responsible for stilbene biosynthesis to produce 1.9 mg/L resveratrol and its mono-methylated derivative pinostilbene (2.4 mg/L) from p-coumaric acid in *E. coli* [37]. In addition to the biosynthesis of natural polyketides, generation of "Unnatural" natural products using "Plug-and-Play" model of synthetic biology was also pursued. Wang,et al. rationally designed a variety of biosynthetic bricks involving totally eight different sources of biosynthetic genes to generate 12 different phenylpropanoids and its derivatives [26]. Type III PKSs were also used to generate small functional molecules in recent years. For instance, Lin and his colleagues firstly designed an artificial *de novo* biosynthesis pathway to generate 4-hydroxycoumarin. A FabH-like quinolone synthase from *Pseudomonas aeruginosa* was employed according to function-based enzyme bioprospecting to enhance the productivity of 4-hydroxycoumarin in *E. coli* [38].

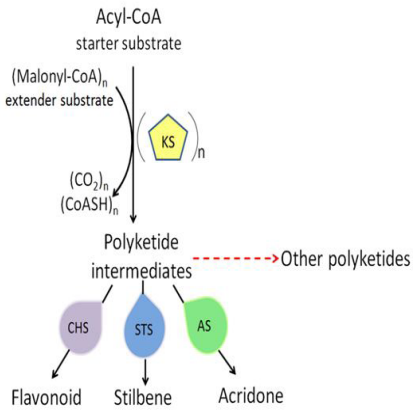

Figure 3: Catalytic mechanism of type III PKSs.Typical type III PKSs use acyl-CoA as the starter unit and malonyl-CoA as the extender unit. KS: ketoacyl synthase; CHS: Chalcone synthase; STS: stilbene synthase; AS: acridone synthase.

Conclusions, General Concerns, and Prospects

Natural products derived from natural sources such as plants, animals and microorganisms are widely used in food, cosmetic, chemical and pharmaceutical industries. Polyketides are a special group of natural products synthesized by PKSs and have remarkable bioactivities. High-cost and laborious physical extraction and complicated multi-step chemical synthesis were commonly used methods to produce them in the past years. In recent years, with the advance of metabolic engineering and synthetic biology, heterologous biosynthesis of polyketides in genetically amendable hosts such as *E. coli* became a promising alternative.

However, economically viable production of these compounds in E. coli has yet to be reached due to the following issues:

1) it is still quite challenging to achieve functional expression of PKSs with high turnover numbers in *E. coli.*

2) acyl-CoA precursors necessary for polyketides formation are usually insufficient to support high-level biosynthesis intracellularly.

3) polyketides can be extremely toxic to *E. coli*hosts due to their strong antibacterial activities.

Past few decades have witnessed a rapid growth of genetic information on PKSs and an improved understanding of the biological and chemical mechanisms of polyketide biosynthesis. All these knowledges provide exciting opportunities for rational design of microbial cell factories for generating more novel polyketides including "Unnatural" ones. In addition, to address the above issues associated with polyketide biosynthesis and achieve efficient production of natural and unnatural polyketides for pharmaceutical industrial applications, novel metabolic engineering and synthetic biology approaches need to be developed. This has already been a major research topic for now and will continue to be an important one in the foreseeable future.

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