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Rational synthetic pathway refactoring of natural products biosynthesis in actinobacteria

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ABSTRACT

Natural products (NPs) and their derivatives are widely used as frontline treatments for many diseases. *Actinobacteria* spp. are used to produce most of NP antibiotics and have also been intensively investigated for NP production, derivatization, and discovery. However, due to the complicated transcriptional and metabolic regulation of NP biosynthesis in *Actinobacteria*, especially in the cases of genome mining and heterologous expression, it is often difficult to rationally and systematically engineer synthetic pathways to maximize biosynthetic efficiency. With the emergence of new tools and methods in metabolic engineering, the synthetic pathways of many chemicals, such as fatty acids and biofuels, in model organisms (*e.g. Escherichia coli*), have been refactored to realize precise and flexible control of production. These studies also offer a promising approach for synthetic pathway refactoring in *Actinobacteria*. In this review, the great potential of *Actinobacteria* as a microbial cell factory for biosynthesis of NPs is discussed. To this end, recent progress in metabolic engineering of NP synthetic pathways in *Actinobacteria* are summarized and strategies and perspectives to rationally and systematically refactor synthetic pathways in *Actinobacteria* are highlighted.

1. Introduction

Natural products (NPs), including antibiotics and other bioactive compounds, are widely used in traditional medicine, especially for treatment of cancer, infectious diseases, cardiovascular disease, and diabetes (Newman and Cragg, 2016). The total annual medical consumption of antibiotics worldwide is estimated at hundreds of thousands of tons (Wise, 2002). *Actinobacteria*, particularly the *Streptomyces* genus, as a high mol% G + C Gram-positive bacteria of terrestrial and marine origin, is used to produce more than 40% of all known microbial bioactive NPs (Bérdy, 2012), and 35% of all marketed antibiotic formulations contain an active ingredient derived from *Actinobacteria* (Gomez-Escribano and Bibb, 2014). During the final decades of the last century, after the “golden age” of antibiotic drug discovery in *Actinobacteria* in the 1980s, only a few antibiotic classes were developed (Hopwood, 2007). However, with the advent of the genomics era, *Actinobacteria* is again regarded as a robust and rich source to search for novel products, as next generation DNA sequencing has revealed a wealth of hidden biosynthetic gene clusters of unknown NPs within the genomes of *Actinobacteria* species (Bentley et al., 2002; Harvey et al., 2015). In addition, with the exploration of marine resources, the marine *Actinobacteria* have also been revealed as huge resources for discovery of new NPs (Gomez-Escribano et al., 2016; Jensen et al., 2015; Subramani and Aalbersberg, 2013; Xiong et al., 2013).

As a microbial cell factory for industrial production of NPs, kinds of irrational and rational approaches have been applied to improve NP titers in *Actinobacteria*. For example, by applying traditional random mutagenesis and screening, a tremendous increase in targeted NP production (such as antibiotics) has been achieved. However, as a time-consuming and labor-intensive approach, random mutagenesis and screening not always enable appearance of the optimal solution in terms of specific mutations that are required for the desirable phenotype (Rokem et al., 2007). In recent decades, with the development of metabolic engineering, *Actinobacteria* have been engineered to produce higher titers of NP. At present, methods to efficiently improve the yield and productivity of NPs derived from *Actinobacteria* are being carefully investigated to reduce energy consumption, as well as

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Table 1
The advantages and challenges of *Actinobacteria* as the microbial cell factory for natural products production.

Advantages
1. **Better suitability or compatibility**
1. High G + C content
2. Support gene expression of mega synthases, *e.g.* polyketide synthase
1. **Powerful platform for natural product biosynthesis**
1. Abundance of intracellular metabolites and intermediates.
2. Delicate structural modification
1. **Performances of resistance**
1. Phage resistance
2. Antibiotics resistances
1. **Mature industrial fermentation and separation techniques...**
Challenges
1. **Low yield or low biosynthetic efficiency of natural products**
1. Complicated transcriptional and metabolic regulations
2. Poor understanding of biosynthetic mechanism
1. **Low efficiency of new structure discovery**
1. Lack of rational guidance
1. **Genetic molecular manipulation**
1. Genetic manipulation of several weeks
2. Number of Actinobacteria are not amenable to genetic manipulation...

waste and emission generation during industrial fermentation in response to a series of published studies showing very high emissions and pollution generated by the manufacture of pharmaceuticals (Larsson, 2014). To this end, more efforts should be taken to maximize the biosynthetic efficiency of the microbial cell factory of *Actinobacteria* by engineering of both the producer and NP synthetic pathway. However, due to the poor understanding of the complicated metabolic and regulatory profiles of targeted synthetic pathways, and limited information on the producers, rational and systematical engineering of synthetic pathways of NPs in *Actinobacteria* is often difficult.

In addition, heterologous expression of genes and gene clusters in *Streptomyces* has been used extensively as a means to access new NPs in the genomics era (Komatsu et al., 2013; Luo et al., 2016). Nevertheless, it is worthwhile to note that occasionally the heterologous production of NPs has very low efficiency or is even unable to produce targeted NPs. In *Streptomyces*, heterologous production of NPs may involve the addition of a precursor and cofactors to regulate transcription of a gene cluster and enzymatic activity of each catalytic protein (Rokem et al., 2007). Therefore, heterologous production of targeted NPs is just a starting point in the drug development process, while the following rational engineering and innovation needed for efficient biosynthesis often remains challenging.

With the emergence of new metabolic engineering methods, the concept of refactoring had expanded strategies to decouple metabolite biosynthesis from complex native regulation or to rewire the NP synthetic pathway. For example, in a model organism, such as *Escherichia coli*, the synthetic pathways of some chemicals, including biofuels and fatty acids, have been systematically refactored to achieve precise and flexible control of production (Xu et al., 2014; Zhang et al., 2012). In *Saccharomyces cerevisiae*, the synthetic pathway of artemisinic acid has also been systematically refactored to achieve highly efficient heterologous production (Paddon et al., 2013). These studies also identified promising approaches for synthetic pathway refactoring in *Actinobacteria*. In this review, the significance of *Actinobacteria* as a microbial cell factory for NP production will be discussed and several challenges that need to be overcome in order to improve biosynthetic efficiency are also considered. To this end, current approaches toward metabolic engineering of NP biosynthetic pathways are briefly summarized. Lastly, a strategy and perspectives to rationally and systematically refactor the NP synthetic pathway in *Actinobacteria* are highlighted.

2. *Actinobacteria* as the microbial cell factory for NP biosynthesis

As the most widely used microbial hosts in metabolic engineering, *E. coli* and *Sa. cerevisiae* have many merits, such as well-understood metabolic backgrounds, amenable genetic manipulation, and fast growth. With the developments in metabolic engineering, many breakthroughs in microbial biosynthesis of various types of pharmaceutical precursors, such as 6-deoxyerythronolide B (Pfeifer et al., 2001), taxadiene (Ajikumar et al., 2010), artemisinic acid (Paddon et al., 2013), and thiamine or hydrocodone (Galanie et al., 2015), have been achieved in these microbes. However, as the producer of most NP antibiotics used clinically today (Lewis, 2013), *Actinobacteria* undoubtedly has more practical significance in pharmaceutical industrial application than *E.coli* or *S. cerevisiae*, as *Actinobacteria*, especially *Streptomyces*, display many unique advantages for NP biosynthesis.

The advantages of *Actinobacteria* as a microbial cell factory for NP biosynthesis are summarized in Table 1. First of all, *Actinobacteria* may be better suited or compatible for the heterologous biosynthetic genes with high G + C content and mega synthases, such as polyketide synthases (PKS) and nonribosomal peptide synthetase (NRPS). It is known that *Actinobacteria* has the most extreme codon usage profile, as most of the wobble positions of the codon are a G or C, resulting in a high G + C content in this organism (*e.g.*, the G + C content in *S. coelicolor* is 71%) (Gustafsson et al., 2004) and the biosynthetic gene cluster with high G + C content from marine *Actinobacteria* could be efficiently heterologously expressed in *Streptomyces* (Yamanaka et al., 2014). In addition, the PKS and NRPS genes of *Actinobacteria* are often composed of tens of kilobase pairs, *e.g.*, in the candicidin (or FR-008) gene cluster and the six genes encoding for type I modular PKS are reported to be as long as 100 kilobase pairs (Chen et al., 2003; Olano et al., 2014). Even so, the large gene cluster (> 80 kb) encoding PKS compounds could also be heterologously produced in *Streptomyces* and other *Actinobacteria* (Huang et al., 2016; Nah et al., 2015; Sosio et al., 2000). Second, *Actinobacteria* is a powerful platform for NP biosynthesis due to its abundance of intracellular metabolites and intermediates, which could be used as precursors for formation of various types of NPs and the great potential for delicate post-modification of chemical structures. As reported by Komatsu et al., more than 20 NPs, which are classified into five classes on the basis of the pathway for generation of corresponding precursors, have been successfully heterologously produced in *Streptomyces avermitilis* (Komatsu et al., 2013). These cases offer a rough idea of the powerful NP biosynthesis capabilities of *Streptomyces*. In addition, the complicated and delicate post-modification capabilities of *Actinobacteria* could also be addressed post-modification during biosynthesis of the type I PKS compound spinosyn in *Saccharopolyspora spinosa* (Mertz and Yao, 1990; Waldron et al., 2001). In the spinosyn biosynthetic gene cluster, 14 of 19 genes are responsible for spinosyn post-modification, which include complicated intramolecular cycloaddition (Fage et al., 2015; Kim et al., 2011) and cross-bridging (Kim et al., 2007), as well as multiple steps of glycosylation (Chen et al., 2009; Hong et al., 2006, 2008; Isiorho et al., 2014; Zhao et al., 2005) and methylation (Kim et al., 2010). After multiple steps of post-modification, these NPs often are too complex for chemical modification. Lastly, as a widely used antibiotic producer in industrial fermentation, *Actinobacteria* have many other meritorious features, such as cognate antibiotic tolerance (Méndez and Salas, 2001), phage resistance (Chinenova et al., 1982), and mature industrial fermentation and separation techniques.

These merits mentioned above render *Actinobacteria* as a suitable host for NP biosynthesis. Hence, many *Streptomyces* spp., such as *S. coelicolor* (McDaniel et al., 1993), *S. avermitilis* (Komatsu et al., 2010), and *S. albus* J1074 (Zaburannyi et al., 2014), have been developed as a universal chassis for the production of heterologous NPs. It is worthwhile to note that *S.* sp. FR-008, which is more amenable to genetic manipulation and fast growth (the doubling time is about 3.6 h), has