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Metabolic engineering of natural product biosynthesis in actinobacteria

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Actinomycetes are known to produce over two-thirds of all known secondary metabolites. We review here recent progress in the metabolic engineering of streptomycetes for natural product biosynthesis. Several examples of the yield improvement of polyketides (mithramycin and tylactone) and non-ribosomal peptides (balhimycin and daptomycin) demonstrate the power of precursor supply engineering. Another example is the manipulation of a regulatory network for increased production of nystatin and teicoplanin. The second part highlights new approaches in the derivatization of natural products via combination of mutasynthesis and genomic engineering.

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Introduction

Actinobacteria, particularly the *Streptomyces* genus, are regarded as a robust source for natural products (NP, or secondary metabolites) discovery. Secondary metabolites from *Actinomycetes* have been successfully implemented in medicine and agriculture due to the broad spectrum of their biological activities, which include antibiotic (teicoplanin), anticancer (mithramycin), immunosuppressive (rapamycin), antiparasitic (avermectin) and many others. However, the identification and development of NP into drugs is a costly and labor-intensive process. Moreover, a very high ratio of rediscovery for known molecules has forced many pharmaceutical companies to change their priorities from NP discovery to target-based screening of synthetic compounds libraries [1]. Therefore, bacteria were considered an already exhausted source for the discovery of new classes of NP. Meanwhile, intensive development and decreasing costs of DNA sequencing and recombinant technologies have revealed a wealth of hidden biosynthetic gene clusters within bacterial genomes [2]. Identification of genes involved in NP biosynthesis and expansion of the genetic toolbox enabled a deeper understanding of the biosynthetic logic of NPs and thus taking it under control [3]. Metabolic engineering, or the introduction of rational changes into the genome of host cells, is a potent approach for the modification and/or improvement of biosynthetic capabilities. During the past several years, metabolic engineering has been successfully applied to numerous clinically important NPs leading to higher titers of the target compound, improvement of desired functions or surprising identification of precursors that are more active than the final molecule [4–6].

Therefore, the two main goals of metabolic engineering are directed toward, first, increasing yields of secondary metabolites and second, derivatization or alteration of NP structure in order to improve their properties. Examples of how these goals can be reached and the genetic tools that have been developed during the past few years are discussed in this review. All natural products discussed in this work are listed in Table 1.

Metabolic engineering for yield improvement

In host cells, secondary metabolites are produced in concentrations that are sufficient for a compound to have a selective advantage for the producer strain (μg-mg/L), as they are not involved in growth. However, these amounts are much less than those necessary for industrial-scale production (g/L). Standard protocols for strain development are based on the selection or screening for the highest-producing native strain and further improving this strain by mutagenesis and selection. With advancements in DNA sequencing and recombinant technologies, rational strain improvement through metabolic engineering became a powerful tool for increasing titers of secondary metabolites. For example, genetic tools can be used to enhance the precursor supply, reducing flux toward competing pathways or unwanted by-products, amendment of gene expression regulation, overexpression of bottle-neck enzymes, amplification of the target gene cluster or heterologous expression of the entire biosynthetic pathway. Typically, the best yields are achieved through a combination of several approaches [7,8].

Precursor supply

The sufficient availability of precursors and intermediates, which are supplied from primary metabolism, is a prerequisite for the biosynthesis and productivity of NPs.

Table 1

List of natural products and approaches that have been used to either increase production yield/titer or for derivatization

Natural product	Approach	Derivative	Productivity increase	Reference
Mithramycin	Carbon flux redirection	–	229%	[7]
Tylactone	Carbon flux redirection, overexpression of pathway-specific regulator	–	10-fold	[8]
Balhimycin	Carbon flux redirection	–	250%	[15]
Daptomycin	Carbon flux redirection	–	30%	[16]
Valinomycin	Carbon flux redirection, deletion of competing pathway	–	4-fold	[18]
Actinorhodin	Amplification of BGC	–	20-fold	[19]
Validamycin A	Amplification of BGC	–	34%	[20]
Chlorotetracycline	Structural gene amplification	–	1.73-fold	[22]
Chloramphenicol	Heterologous expression[a]	–	10-fold	[27]
Phloroglucinol	Heterologous expression[a]	–	4-fold	[28]
4-Hydroxycoumarin	Heterologous expression[a]	–	2.53-fold	[29]
Resveratrol	Heterologous expression[a]	–	1.70-fold	[29]
Naringenin	Heterologous expression[a]	–	1.53-fold	[29]
Teicoplanin	Overexpression of pathway-specific regulator	–	40-fold	[32]
Simocyclinone	Overexpression of pathway-specific regulator	–	2.5-fold	[33]
C-1027	Overexpression of pathway-specific regulator	–	5-fold	[34]
Tetramycin A	Overexpression of pathway-specific regulator	–	3.3-fold	[35]
Nystatin A1	Deletion of pathway-specific regulator	–	2.1-fold	[35]
Kinamycin	Deletion of pathway-specific regulator	–	Identification[b]	[36]
Taromycin	Deletion of pathway-specific regulator, heterologous expression[a]	–	Identification[b]	[37]
Moenomycin	Overexpression of pleiotropic regulator	–	2.5-fold	[38]
FK506	Structural genes amplification	–	146%	[21]
Cinnabaramide	Mutasynthesis	Cyclopentylcinnabaramide, chlorocinnabramides	–	[39]
FK506	Mutasynthesis	Exclusive production, 31-desmethoxytacrolimus, TC-255	–	[42,21]
Rapamycin	Mutasynthesis	39-desmethoxyrapamycin, BC346, BC347	–	[11]
Erythromycin	Site-directed mutagenesis of AT domains	Allylerythromycin, propargylerythromycin, ethylerythromycin	–	[44,45]
Clorobiocin	Biosynthetic engineering	Novclobiocin 401	–	[41]
Tetracenomycin	Biosynthetic engineering	Ketoolivosyl-tetracenomycin C	–	[46]
Spectinabilin	Plug-and-play strategy	–	Identification[b]	[55]
PTM	Plug-and-play strategy	–	Identification[b]	[54,56]

[a] Heterologous expression in genetically engineered host.
[b] Identification refers to compound not detected or detected in minor quantities in the wild type strain

Optimization of media components may lead to two-fold to seven-fold increase in production [9,10] however, classical strain development [11] and genetic engineering [8,12] typically are more effective and can increase production up to 10 times.

There are two ways to increase the intracellular concentration of building blocks: elimination of competing pathways [13] and overexpression of appropriate biosynthetic genes [14]. Malonyl-CoA (MCoA), an important building block for the biosynthesis of polyketides, is derived from the carboxylation of acetyl-CoA by the acetyl-CoA carboxylase complex (Acc-ase) (Figure 1). The manipulation of MCoA biosynthesis led to significant increase of the mithramycin yield. Mithramycin is an antitumor compound with a polyketide core and two sugar and two aliphatic side chains,