

Approaches of Genetic Regulation in Actinomycetes for Antibiotic Synthesis

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Description

Microorganisms, especially actinomycetes, can generate a wide variety of bioactive secondary metabolites [1]. The majority of these metabolites have positive effects on antibiosis, anti-tumor, pesticide, immunosuppression and immune activation, and they therefore have been widely applied in medicine, agriculture and food industry [2]. With the appearance of drug-resistance bacteria, many researches today are focusing on how to improve the biosynthesis of antibiotic in actinomycetes and how to exploit new antibiotics [3]. However, the most effective way to achieve this is by regulating the process of secondary metabolism in genetic levels [4,5]. Therefore, this overview will summarize current approaches of genetic regulation in the antibiotic synthesis of actinomycetes.

Regulation of Expression of Regulatory Genes

The genes responsible for antibiotic biosynthesis generally exist as a cluster in actinomycetes [6]. In addition to structural genes, these gene clusters usually contain regulatory genes, resistance genes against corresponding antibiotics and transport genes [7]. And the antibiotic biosynthesis is therefore simultaneously controlled by these resistance genes, regulatory genes and transport genes [8]. However, the transcription of gene clusters relies mainly on the regulations of general or specific regulatory proteins [9,10]. Generally, specific regulatory genes exist in the cluster relating to the biosynthesis of secondary metabolites, like *Streptomyces* antibiotic regulatory protein (SARP) [10,11]. Also, the specific regulation involves the positive regulation and negative regulation [8]. Therefore, the biosynthesis can be increased by over-expressing the positive regulatory genes and knocking off the negative regulatory genes.

Regulation of expression of Gene Clusters

Two approaches contribute to this regulation. One is to increase the number of copies of gene clusters responsible for antibiotic biosynthesis [12]. This means that increasing the copies of gene clusters can directly manage to improve the biosynthesis when the relevant gene clusters have already been known [12-14]. However, this method has its shortage that the increase of production is limited because of the influences from other genetic regulations. Moreover, the other approach is by controlling the heterogeneous expression of gene clusters relating to antibiotic synthesis [15,16]. There are a huge variety of actinomycetes in the nature, but only 10% of them have been exploited for antibiotic production [17,18]. Genome mining could recognize gene clusters relating to antibiotic biosynthesis via bioinformatic technology and then activate the relevant gene clusters

through genetic engineering [19]. Combining with genome mining, the heterogeneous expression could come true by introducing a wide range of wild-type genes. Therefore, it is also an effective approach to develop new antibiotics.

Regulation of Expression of Resistance Genes

Resistance genes determine the level of antibiotic resistance in actinomycetes [19]. Resistance proteins will inhibit the expression of relevant genes and subsequently prevent the biosynthesis of antibiotic if the majority of resistance proteins react with antibiotics resulting in the concentration of antibiotic exceeding the resistance of cell [20]. Therefore, improving the expression of resistance genes or the tolerance to antibiotic in actinomycetes can increase the biosynthesis of antibiotic [18].

Regulation of Expression of Transport Genes

It is well reported that both growth and metabolism of cells will be inhibited if the concentration of intracellular antibiotics reach beyond the level of resistance of cells [21]. Therefore, pumping out antibiotics timely from cells and maintaining antibiotics at the normal concentration can greatly increase their biosynthesis in actinomycetes [22]. However, transport proteins play a key role in this process. Accordingly, we can then improve the biosynthesis through the expression of relevant transport genes.

Regulation of Expression of Ribosome-related Proteins

By now, it is reported that three main synthetic pathways, namely PKS, NRPS and RiPPs, contribute to the biosynthesis of antibiotic [23,24]. Among them, RiPPs must be completed in the ribosome and therefore some ribosome-related proteins would have effects on the biosynthesis of antibiotic. Ribosome recycling factor (RRF) is the protein that takes charge of dissociating the relevant RNA from ribosome when the transcription is completed [25,26]. For instance, over-expressing the gene *frr* in *Streptomyces diastatochromo*-genes 1628 has managed to increase the production of antibiotic [27,28]. RimP-SC is an assembly protein in ribosome, and knocking off this RimP-SC gene could increase the biosynthesis of antibiotic Act and CDA in *Streptomyces coelicolor* [29]. Likewise, in *Streptomyces venezuelae*, the biosynthesis of jadomycin could be improved by knocking off RimP-SV genes [30].

There are many unknown gene clusters that take responsibility for the biosynthesis of secondary metabolites in actinomycetes. However, these gene clusters usually have low expression or even no expression in trial. Also, many valuable secondary metabolites than can be used as

the source of new drugs exist in actinomycetes. With the development of bioinformatics, researches on antibiotics will emphasize on how to explore and recognize secondary-metabolite-related gene clusters, then how to transform and assemble the recognized clusters via DNA engineering, and finally how to heterologously express and activate these clusters. Besides, the metabolic regulation is also an effective approach to improve the biosynthesis of antibiotic in actinomycetes. In summary, genetic regulation would have more potential and promise in the improvement and development of antibiotic industry.

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