



Research Highlights

Unlocking a new target for streptomycetes strain improvement

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ARTICLE INFO

Keywords:

Polyketide

Streptomyces

Strain Improvement

Triacylglycerol

 β -oxidation

Acetyl-CoA

Bacteria of the *Streptomyces* genus are well-known producers of secondary metabolites of high medical value. They contributed nearly 60% of current antibiotics (i.e., vancomycin, daptomycin and tetracycline), as well as antifungals, antiparasitics (ivermectins), anticancer drugs (doxorubicin), immunosuppressants and others [1]. Therefore, in the past several decades, *Streptomyces* bacteria or the therein involved biosynthetic pathways have long been the central topic of strain improvement, metabolic engineering, and bioengineering. Much of this research has focused on regulatory elements; however, it has been a challenge due to the complex regulatory network that controls *Streptomyces* secondary metabolism and its complex fungus-like morphological differentiation [2]. On agar plates, production of secondary metabolites coincides with the switch from vegetative growth (substrate mycelium) to aerial mycelium hyphae and subsequent spore formation, whereas in a liquid culture, early stationary phase when cells stop growing marks the initiation of secondary metabolism.

In addition to regulatory targets, others have attempted to re-wire the energy flux to direct more substrates from primary metabolism to secondary metabolism. All secondary metabolites are built upon substrates from the primary metabolism, such as acetyl-CoA, amino acids, isoprenoids, or lipids. A groundbreaking work from Drs. Lixin Zhang and Wensheng Xiang laboratories was recently published in Nature Biotechnology [3], in which they unambiguously demonstrated triacylglycerols (TAGs) as the carbon source for polyketide production in *Streptomyces coelicolor* A3(2), the model strain of the genus.

Polyketides, a large group of structurally diverse secondary metabolites found in streptomycetes and many other microorganisms, are

produced by polyketide synthases (PKSs) in an assembly line-like manner which incorporates a two-carbon unit per reaction or elongation step [4]. Acetyl-CoA and its derivative malonyl-CoA are often the starting unit and the extension substrate, respectively. Acetyl-CoA, a product of glucose catabolism, is a central substrate for almost all primary metabolic processes, such as the TCA cycle and lipid synthesis (Fig. 1), it also serves as a major precursor for secondary metabolism. For a long time, sugar (i.e., glucose) has been regarded as the carbon source for polyketide biosynthesis, although few studies suggested a potential link between lipid degradation and antibiotic production, such as spiramycin [5] and actinorhodin (ACT) [6,7]. Unlike other bacteria, TAGs is the major form of lipids accumulated in *Streptomyces* strains, accounting for up to 60% of total lipids during the late stationary phase [8,9]. The *S. ambifaciens* mutant NSA205 (ADS205⁺, Spi⁻), which harbors an amplified DNA sequence ADS205, does not produce spiramycin and is largely depleted in the production of both phospholipids and TAGs. Its derivative NSA228 (ADS205⁻, Spi⁺) which lacks ADS205 restored spiramycin production. Authors also founded that the lipid production in NSA228 was globally restored to wild-type levels, indicating a potential role of lipids in antibiotic production [5]. An open reading frame *orfPS* in ADS205 likely encodes a PKS. Amplification of *orfPS* in NSA205 may cause competition for the same precursor acetyl-CoA between fatty acid synthesis or other PKS pathways. Via the studies of *ppk* mutants, a similar role of TAGs was also noted for the production of the blue-pigmented polyketide antibiotic ACT in *S. coelicolor* and *S. lividans* [6,7]. *Ppk* modulates the ATP/ADP metabolism in streptomycetes acting either as a polyphosphate kinase (ATP

Peer review under responsibility of KeAi Communications Co., Ltd.

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Received 30 January 2020; Accepted 3 February 2020

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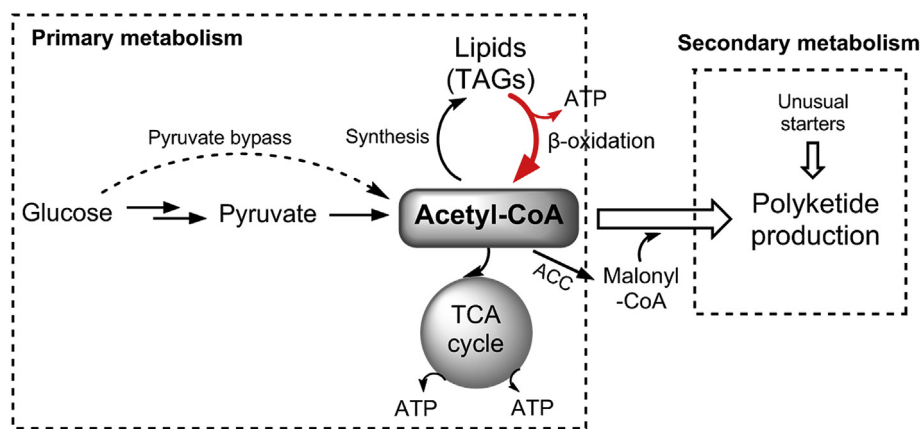


Fig. 1. Acetyl-CoA catabolism in primary and secondary metabolisms.

degradation) or as an adenosyl diphosphate kinase (ATP generation) depending on the ATP/ADP ratio. In *ppk* mutants, TAG degradation was triggered by energetic stress. The expression of β -oxidation enzymes such as SCO3051 (acyl-CoA dehydrogenase) and SCO5144 (enoyl-CoA hydratase) was elevated, generating a flux of acetyl-CoA toward the production of ACT. Conversely, wild type *S. lividans*, a weak producer of ACT, accumulated a high level of TAGs [6].

However, no direct evidences, nor detailed mechanisms, have been reported for the role TAGs in polyketide production. In Zhang and his colleagues' work, they unambiguously demonstrated that, via comparative metabolome analysis of *S. coelicolor* A3(2) wild-type M145 and a mutant HY01 overproducing ACT, carefully designed glucose- and oleate-isotope labelling and feeding experiments, detailed energy flow analysis, and transcriptome analysis, acetyl-CoA for polyketide production is degraded from TAGs. Results indicated glucose is almost used up at the end of primary metabolism, while the accumulation of TAGs is at its peak. Once entering early stationary phase when ACT production starts, TAGs levels declined. Unlike previous studies, authors identified SCO6196, a fatty acyl-CoA synthase, as the key enzyme that degrades TAGs in *S. coelicolor*. This direct relationship was further confirmed by knock-out and overexpression of the gene *SCO6196*. The production of ACT is almost diminished in the *SCO6196* knock-out mutant which accumulated high levels of TAGs. On the contrary, overexpression of *SCO6196* enhanced ACT production from nearly 80 mg/L to 120 mg/L, accompanied by a very low level of TAGs in the recombinant strain [3].

Ultimately, a dynamic degradation of TAG (ddTAG) strategy, which enables a finely controlled induction of *SCO6196* using a cumate-inducible promoter, was developed. With this strategy, the titer of ACT reached an astonishing level of 216 mg/L, 190% of ACT produced in the wild-type M145. Most excitingly, the ddTAG strategy worked very well in other *Streptomyces* strains via the induction of *SCO6196* homologs, such as in the producers of jadomycins, oxytetracycline and avermectin B_{1a} [3]. It is notable that the induction of avermectin B_{1a} production was done in an industrial scale 180-L fermenter, achieving an incredible yield of 9.31 g/L, which is a 50% increase compared to the non-induced culture. Interestingly, by perturbing the β -oxidation pathway in yeast *Yarrowia lipolytica*, the heterologous production of a simple yet

industrially important polyketide triacetic acid lactone (TAL) and 4-coumaroyl-CoA derived polyketides bisdemethoxycurcumin, naringenin and resveratrol was significantly enhanced [10]. These results indicate lipid catabolism (i.e., β -oxidation pathway) is an efficient target for strain improvement in both bacteria and fungi, in particular for increasing polyketide production.

Acknowledgments

This article was supported in part by the Faculty Excellence support from the Harbor Branch Oceanographic Foundation.

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