



Research Highlights

Unleashing the power of energy storage: Engineering β -oxidation pathways for polyketide production

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ABSTRACT

Overproduction of polyketides has been a challenge for metabolic engineering for decades. However, recent studies have demonstrated that in both native host and heterologous host, engineering β -oxidation pathways can lead to dramatic improvement of polyketide production.

Polyketides are a family of structurally diverse secondary metabolites produced by bacteria, fungi and plants, many of which have important biological activities. Despite their vast structural diversity, they are biosynthesized by only three types of polyketide synthases via successive condensation of simple building blocks, such as acetyl-CoA and malonyl-CoA. Therefore, employing metabolic engineering strategies to improve their titers for medical and industrial applications is of great interest.

Neutral glycerides, specifically triacylglycerols (TAGs), are highly dense energy molecules that are commonly stored in eukaryotes as lipid bodies. Yet their surprising presence, sometimes at an even substantial fraction, in the cells of prokaryotic streptomycetes was soon correlated to the secondary metabolism and polyketide production in early 1980s [1,2]. This correlation remained unclear for decades due to the complex morphological and physiological differentiations that are often accompanied by a dramatic metabolic switch from central carbon primary metabolism to specialized secondary metabolism [3,4]. However, this was completely changed by the recent work published in *Nature Biotechnology* by Zhang and co-workers who unambiguously established the correlation between storage TAG degradation and polyketide production by extensive multi-omics analyses coupled with genetic manipulations [5]. More significantly, this discovery was successfully transferred to practical applications.

Zhang and co-workers showed that the lipid pathway metabolites, discriminately the storage TAGs, were accumulated in the early stages

of cell growth and reached the maximum in the post-exponential phase, and then declined continuously afterward. This trend is well consistent with the time course of the biosynthesis of polyketide actinorhodin (Act). Supported by transcriptomic analysis, they found genes involved in fatty acid biosynthesis were downregulated in the stationary phase, in clear contrast with the β -oxidation pathway genes for TAG degradation that are upregulated when Act is produced. Yet the direct evidence was from detailed comparative investigation of the Act wild type producer and a high Act producing strain as well as other three polyketide producing industrial strains. These results showed a remarkable proportion of cellular TAG was degraded in the stationary phase and the resulting acetyl-CoA and reducing power further depressed the Krebs cycle and diverted the carbon flux to the polyketide biosynthesis. Fatty acyl-CoA synthetase (ACS) is the first committed step involved in activation of free fatty acid in the TAG degradation process. Simply overexpressing the ACS gene substantially increased the Act production (Fig. 1). To maximize the polyketide production, they followed the cell growth curve and devised a dynamic TAG degradation module to control the timing and strength of ACS expression, which was successfully applied to several important polyketide compounds either from the parental or industrial strains. Among them a 50% titer improvement (from 6.20 to 9.31 g L⁻¹) was obtained for an industrial avermectin B_{1a} producer strain in a 180-m³ stirred-tank bioreactor, indicating the vast potential of this strategy for improving the polyketide titers in *Streptomyces*.

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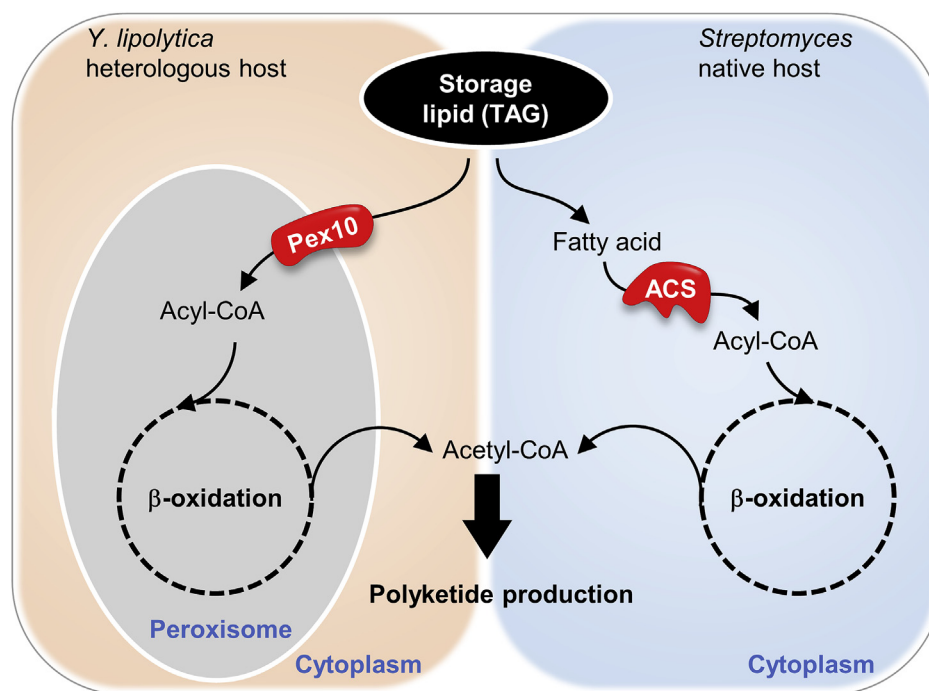


Fig. 1. Metabolic engineering of β -oxidation pathways in *Y. lipolytica* and *Streptomyces* hosts for polyketide production. Abbreviations: TAG, triacylglycerol; ACS, fatty acid CoA synthetase.

Coincidentally, two recent papers published by the Alper group explored the same β -oxidation pathway in oleaginous yeast *Yarrowia lipolytica* to achieve high-level polyketide production [6,7]. Although lipid bodies are substantially accumulated in yeast, it was shown that disrupting the genes involved in the synthesis and degradation of storage lipids such as TAGs, steryl esters, and even the lipid bodies themselves, is non-detrimental to yeast cells [8]. This finding generated great interests in engineering yeast cells for production of fatty acid derived chemicals and biofuels [9,10], and in a few cases polyketides.

Alper and coworkers first attempted to engineer *Y. lipolytica* for high level production of triacetic acid lactone (TAL), a simple polyketide but a platform chemical for a myriad of valuable products. They investigated three distinct pathways in the starting strain YT (*Y. lipolytica* containing *Gerbera hybrida* 2-pyrone synthase gene, *g2ps1*) to promote the supply of precursors, acetyl-CoA and malonyl-CoA, including the pyruvate dehydrogenase complex pathway, the pyruvate bypass pathway, and the β -oxidation pathway (the resulting strain was named YT-PEX10) (Fig. 1). Among them, the pyruvate bypass pathway engineering achieved an unprecedented titer of 36 g L^{-1} in a bioreactor. However, it can only be accomplished in complex medium rather than the defined medium, impeding practical industrial application. In a follow-up study, Alper and coworkers continued the engineering efforts with YT-PEX10. Further overexpression of the native acetyl-CoA carboxylase gene *ACC1*, dramatically improved the TAL titers from 2.4 to 8.6 g L^{-1} in defined medium in a bioreactor. More encouragingly, they extended their work to synthesize 4-coumaric acid *de novo* by a heterologous pathway, and combined with deregulation of amino acid biosynthesis, they accomplished production of naringenin, resveratrol, bisdemethoxycurcumin, and (*E*)-5-(4-hydroxyphenyl)-3-oxopent-4-enoic acid, among which naringenin was produced up to 898 mg L^{-1} , the highest titer ever reported in a microbial host.

In conclusion, the past decades have witnessed the marvelous development of polyketides as either pharmaceutical agents or industrial chemicals, but such development heavily relied on genetic engineering of genes or operons confined to the biosynthetic gene clusters and only low titers of polyketides were obtained in most cases. The studies by Zhang and coworkers and Alper and coworkers have demonstrated that

in both native host and heterologous host, metabolic engineering of β -oxidation pathways can lead to dramatic improvement of polyketide production, which represents a new powerful strategy for exploiting polyketides for basic and applied research.

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