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Spotlight

Growth-coupled evolution accelerates efficient biosynthesis of natural products

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Microbial natural product biosynthesis often suffers from metabolic burden and genetic instability. Bushin *et al*. introduce a synthetic C1 feedback loop that couples xanthommatin production to cell survival, enabling adaptive laboratory evolution to achieve gram-scale production. This strategy offers a generalizable route to engineer cell factories for complex natural products.

Commercial production of many natural products has long relied on extraction from native organisms—a process constrained by low yields, high costs, limited sustainability, and substantial land and labor demands. Synthetic biology and metabolic engineering have emerged as sustainable alternatives, enabling several successes in commercial-scale bio-based production of value-added products [1,2]. However, the complex, enzyme-rich pathways required for many natural products impose significant metabolic burden and often exhibit inefficient metabolic flux, necessitating hundreds of person-years of effort for strain development. Furthermore, as such complex pathways often impose a growth burden, loss-of-function mutants with reduced production readily outcompete high-performance strains, resulting in genetic instability and declining productivity.

One way to counter this evolutionary disadvantage is to couple product formation to cell growth by integrating the target biosynthetic pathway with an essential metabolic function [3,4]. In this growth-coupling scheme, cells must produce the target molecule to sustain growth, thereby aligning product formation with cellular fitness and enabling natural selection to enrich high-performing producers. Since previously reported growth-coupling strategies linked the target product or their intermediates to primary metabolism, carbon flux and cellular energy were diverted toward biomass formation and maintenance rather than product biosynthesis, leading to suboptimal product yields [5,6]. To address this challenge, Bushin and colleagues report an alternative growth-coupling strategy that employs the byproduct formate as the central metabolite linking host primary metabolism to the heterologous biosynthetic pathway for the target product (Figure 1A) [7]. This study also presents the first report of microbial production of the animal pigment xanthommatin (Xa), achieving gram-scale titers in fed-batch fermentation.

Xa is an ommochrome pigment derived from L-tryptophan via the kynurenine pathway. It is abundant in cephalopods and arthropods, where it contributes to eye and skin pigmentation. Beyond its biological functions, Xa displays strong ultraviolet absorption and antioxidant properties, making it an attractive candidate for industrial biomaterials, including photoelectronic devices, dyes, and ultraviolet-protective agents. Although several studies have reported chemical routes for Xa synthesis, these methods are limited by sustainability concerns and the high cost of precursors [8].

For sustainable and efficient Xa production from renewable, nonedible biomass, *Pseudomonas putida* was selected as the chassis due to its high tolerance to toxic compounds such as formic acid and Xa, as well as its intrinsically robust metabolism toward L-kynurenine, the immediate precursor of Xa. First, the Xa biosynthetic pathway was constructed to convert L-tryptophan to L-3-hydroxykynurenine through the sequential reactions of tryptophan 2,3-dioxygenase, kynurenine formamidase (KFA), and kynurenine monooxygenase, all derived from *Pseudomonas* sp. DTU12.1, followed by oxidative dimerization—likely catalyzed by endogenous catalases—to yield Xa. Importantly, formate is generated as the byproduct during the conversion of *N*-formyl-L-kynurenine to L-kynurenine by KFA. To create a growth-coupled production host, the authors engineered the platform strain (PUMA) to be auxotrophic for the essential metabolite 5,10-methylenetetrahydrofolate by deleting two *glyA* copies and the two *gcvTHP* operons and then introducing a formate assimilation pathway to render its growth dependent on formate. The Xa biosynthetic pathway was then introduced into PUMA to generate the PUMA-Xa strain, in which formate released from Xa biosynthesis restores the engineered auxotrophy, thereby directly coupling Xa biosynthesis to cell growth under glycine and tryptophan supplementation. By enabling a heterologous secondary metabolite pathway to rescue an essential primary metabolic function, the study effectively embeds Xa biosynthesis into the core metabolic network—placing the pigment and the host inextricably 'in the same boat' (Figure 1B).

To enable Xa production without the need for external glycine and tryptophan, Bushin and colleagues applied a robotic platform-assisted adaptive laboratory evolution (ALE) to the PUMA strain through gradual amino acid weaning. During serial subcultures, glycine supplementation was gradually reduced and eliminated, followed by the stepwise withdrawal of tryptophan. The resulting evolved strain,