Producing the Polyketide Antibiotic Actinorhodin From Azo Dye Waste: In silico Design and Optimization of a Synthetic Pathway in Pseudomonas putida KT2440

Nayyara, Parsa (School: SMA Negeri 5 Surabaya)

Recent advances in synthetic biology have enabled the conversion of industrial wastes into high-value chemical products, including specialized metabolites such as polyketides. Azo dyes, a major pollutant commonly discharged by food, textile, and pharmaceutical industries, have the potential to be repurposed as a feedstock for biomanufacturing. Here, I sought to design a synthetic pathway in Pseudomonas putida KT2440 capable of recycling azo compounds into the polyketide actinorhodin (ACT). The synthetic pathway was constructed by combining a set of reactions identified from publicly-available databases. Flux balance analysis was used to test the metabolic potential and limitations of the synthetic pathway in P. putida using a modified version of the iJN1463 genome-scale metabolic model. The algorithms Optknock, Optgene, and FSEOF were employed to examine whether coupling dye consumption and ACT production with growth could be a suitable optimization strategy. It was revealed that while dye degradation is always coupled with growth, growth-coupling for ACT production is theoretically inefficient. Thus, a synthetic genetic circuit was constructed to decouple growth and ACT production in a two-stage fermentation scenario. Five refactored operons were designed to modularize the ACT gene cluster and avoid overlapping of native and synthetic regulatory elements. To express the system in P. putida, the regulatory circuit and refactored operons were integrated into pRO1600 Pseudomonas-specific and pBBR1 broad-host-range plasmids using Gibson Assembly. This research demonstrates that the conversion of azo dyes into actinorhodin in P. putida is feasible. Moreover, this work establishes a systematic workflow and testable genetic designs to be further investigated experimentally.

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