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Determination of the Rate-Limiting Step in Post-PKS Modification Pathway and Enhanced Production of Hydroxylated Macrolides

Sang Kil LEE[1], Jay Sung Joong HONG[1], Won Seok JUNG[1], Cha Yong CHOI[1], Jong Seog AHN[2], Sung Ryeol PARK[3] and Yeo Joon YOON*

Nano Sciences and Department of Chemistry, Ewha Womans University, Seoul, 120-750.
[1]School of Chemical and Biological Engineering, Seoul National University, Seoul, 151-742.
[2]Korea Research Institute of Bioscience and Biotechnology, Daejeon, 305-333.
[3]Nano Sciences, Ewha Womans University, Seoul, 120-750.
**Corresponding author: joonyoon@ewha.ac.kr*

The post-PKS modification reactions including hydroxylation step catalyzed by cytochrome P450 monooxygenases, are often crucial to structural diversity and biological potency of macrolide polyketides. Here, we suggested a metabolic engineering strategy to determine the rate-limiting step during macrolide polyketide biosynthesis and enhance the productivity of the desired hydroxylated polyketides. In the liquid culture of *Streptomyces venezuelae*, intermediate macrolides accumulate and only a small amount of the last-stage hydroxylated compounds is produced. Improved production of hydroxylated polyketides mediated by the PikC hydroxylase from *S. venezuelae* was achieved by overexpression of *pikC* gene, supplementing ferrous sulfate into the liquid medium, and feeding of aglycones. In the liquid culture of the mutant strain (YJ029) overexpressing *pikC* gene, the bioconversion of 12-membered ring macrolide YC-17 to (neo)methymycin increased about three folds as compared with that of wild-type. In the case of 14-membered ring macrolide narbomycin, the production of pikromycin increased about five-fold. In addition, adding ferrous sulfate into the medium and feeding of aglycones in the culture of YJ029 resulted in a significantly higher production of the desired hydroxylated products