Generation of hybrid polyketide by flexible hydroxylase from pikromycin biosynthesis cluster in *Streptomyces venezuelae*

이상길[1], 김정호[2], 유영신[2], 홍성중[1], 정원석[1], 최차용[1], 윤여준[2],
[1]서울대학교 응용화학부 생물공학실험실, [2]울산대학교 생명화학공학부 유전공학실험실
전화 (052) 259-2523, FAX (052) 259-1689

Abstract

The biological activity of many polyketide antibiotics requires the presence of one or more hydroxy groups attached to aglycone. And the biological importance of the resulting hydroxyl substituents to many macrolide antibiotics compounds is often represented by a significant increase in antibiotic potency.[1)] Therefore, the properties of existing polyketides would be changed by conferring hydroxy group on another position of them. A method was developed for the generation of hybrid polyketide by the bioconversion of authentic antibiotic into its altered form through attaining the hydroxy group at a certain position. Polyketide cytochrome P450 monooxygenases catalyze the site-specific oxidation of the precusors to many macrolide antibiotics. These reactions occur in the late stages of biosynthesis after formation of the macrocycle by the polyketide synthase (PKS).[2),3)] Among these enzymes, cytochrome P450 hydroxylase (PikC) from pikromycin biosynthesis cluster in *Streptomyces venezuelae* mediates the hydroxylation of macrolactones YC-17 and narbomycin to 12-membered macrolides (methymycin and neomethymycin) and the 14-membered macrolides (narbomycin and pikromycin), respectively (Figure 1).[1),4)] This substrate flexibility is unique and represents the first example of a P450 hydroxylase that can accept 12- and 14-membered ring macrolides as substrates, as well as functionalize at two positions on the macrolactone system.[4)] This broad substrate specificity of PikC provides us with a potentially valuable tool in the production of novel hybrid polyketides.[5),6),7)] Feeding heterologous polyketides to the engineered mutant of *Streptomyces venezuelae*, in which the last module of PKS, *pikA IV*, is deleted and so antibiotics production is prohibited, we have shown the possibility that *pikC* could catalyze the hydroxylation of alternative substrates. The 14-membered macrolide polyketide oleandomycin, which structurally resembles pikromycin and narbomycin, has been expected

to be converted into its hydroxylated form by the bioconversion method (Figure 2).

The hydroxylated form of oleandomycin has been detected primarily by thin layer chromatography and high-perfomance liquid chromatography. These results suggest that a wide variety of macrolide polyketides should be accessible by this approach.

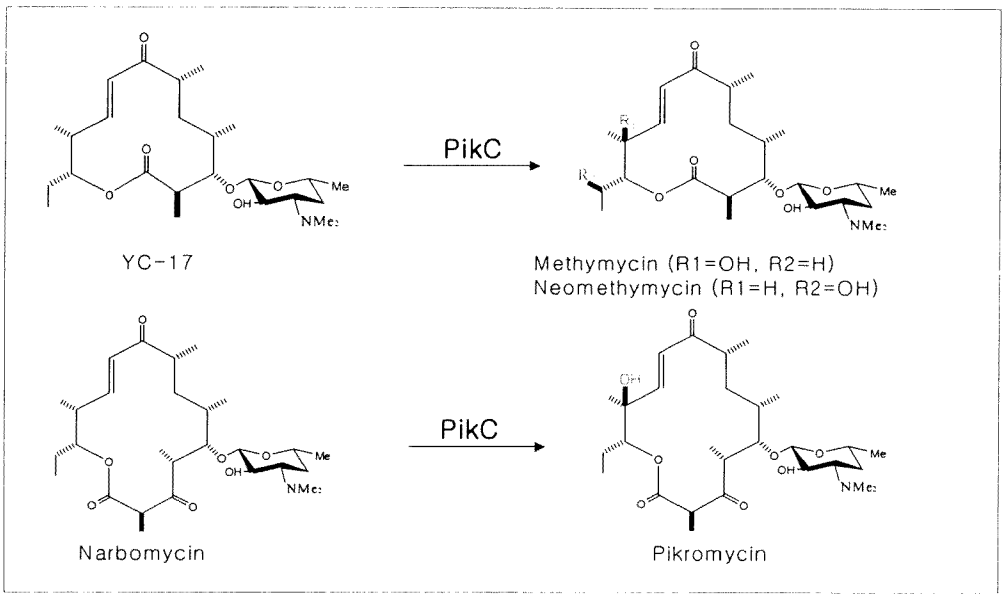

Figure 1. Conversion of YC-17 and narbomycin into their related antibiotics by PikC

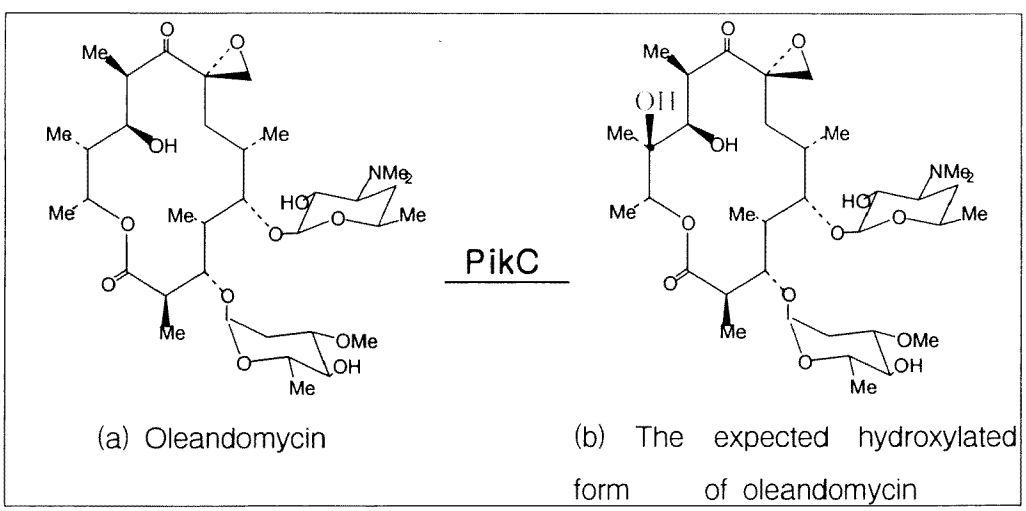

Figure 2. The structure of oleandomycin(a) and the expected hydroxylated form of oleandomycin by PikC(b)

References

1. Melanie C. Betlach, James T. Kealey, Mary C. Betlach, Gary W. Ashley, & Robert McDaniel, "Characterization of the Macrolide P-450 Hydroxylase from *Streptomyces venezuelae* Which Converts Narbomycin to Picromycin" (1998), *Biochemistry*, 37, 14937-14942
2. Weber, J. M., Leung, J. O., Swanson, S. J., Idler, K. B., & McAlpine, J. B., "An erythromycin derivative produced by targeted gene disruption in *Saccharopolyspora erythraea*" (1991) *Science*, 252, 114-117
3. Uwe Rix, Carsten Fischer, Lily L. Rimsing, & Jurgen Rohr, "Modification of post-PKS tailoring steps through combinatorial biosynthesis" (2002), *Nat. Prod. Rep.*, 19, 542-580
4. Yongquan Xue, Daniel Wilson, Lishan Zhao, Hung-wen Liu, & David H Sherman, " Hydroxylation of macrolactones YC-17 and narbomycin is mediated by the *pikC*-encoded cytochrome P450 in Streptomyces venezuelae" (1998), *Chemistry & Biology*, 5, 661-667
5. Yeo Joon Yoon, Brian J. Beck, Beom Seok Kim, Han-Young Kang, Kevin A. Reynolds, & David H. Sherman, "Generation of Multiple Bioactive Macrolides by Hybrid Modular Polyketide Synthases in *Streptomyces venezuelae*" (2002), *Chemistry & Biology*, 9, 203-214
6. Brian J. Beck, Yeo Joon Yoon, Kevin A. Reynolds, & David H. Sherman, "The Hidden Steps of Domain Skipping: Macrolactone Ring Size Determination in the Pikromycin Modular Polyketide Synthase" (2002), *Chemistry & Biology*, 9, 575-583
7. Yongquan Xue & David H. Sherman, "Biosynthesis and Combinatorial Biosynthesis of Pikromycin-Related Macrolides in *Streptomyces venezuelae*" (2001), *Metabolic Engineering*, 3, 15-26