Engineering the deoxysugar moiety of polyketides in *Streptomyces venezuelae*

Yeo Joon Yoon

Division of Nano Sciences and Department of Chemistry, Ewha Womans University

11-1 Daehyun-dong, Seodaemun-gu, Seoul, 120-750, Korea

Email: joonyoon@ewha.ac.kr, Tel: +82-2-3277-4082, Fax: +82-2-3277-3419

Several strategies using combinatorial biosynthesis were employed to produce polyketides with altered glycosylation pattern in *Streptomyces venezuelae* ATCC 15439. This wild type strain was manipulated by deleting different portions of the pikromycin genetic cluster to produce mutants that produce only the aglycon (10-deoxymethynolide and narbonolide), the deoxysugar (TDP-D-desosamine) or a completely deleted strain that is a non-producer, which were named YJ003, DHS2001 and YJ028, respectively. Firstly, a two plasmid system expressing the genes for the biosynthesis and transfer of exogenous deoxysugars, TDP-D-quinovose or TDP-D-olivose was incorporated into the strainYJ003 to produce novel quinovose or olivose glycosylated 10-deoxymethynolide and narbolide. Secondly, expression of the tylactone PKS from another two plasmid system in the strain DHS2001 led to the production of desosamine glycosylated tylactone. Thirdly, feeding of tylactone to the strain YJ028 that was transformed by plasmids expressing the TDP-quinovose or TDP-olivose biosynthetic and transfer genes led to the production of quinovose glycosylated or olivose glycosylated tylactone. In all these systems, an auxiliary protein besides the glycosyltransferase was found to be essential for glycosylation to proceed efficiently. The pairing of glycosyltransferase and auxiliary protein in glycosylation was further examined by deleting the gene encoding the auxiliary protein (DesVIII) from the wildtype *S. venezuelae* and complementationby exogenous auxiliary proteins from other polyketide gene clusters. These results demonstrate a successful attempt of genetically engineering the *S. venezuelae* for the generation of novel hybrid macrolide antibiotics.

Recent publication

1. Lee, S. K., Basnet, D. B., Choi, C. Y., Sohng, J. K., Ahn, Jong Seog, and Yoon, Y. J., "The Role of Erythromycin C-12 Hydroxylase, EryK, as a Substitute for PikC Hydroxylase in Pikromycin Biosynthesis," **Bioorganic Chemistry**, 2004, 32(6), 549-559.
2. Jay Sung Joong Hong, Sung Hee Park, Cha Yong Choi, Jae Kyung Sohng , Yeo Joon Yoon, "New olivosyl derivatives of methymycin/pikromycin from an engineered strain of Streptomyces venezuelae," **FEMS Microbiology Letters**, 2004, 238(2), 391-399.
3. Sang Kil Lee, Devi B Basnet, Jay Sung Joong Hong, Won Seok Jung, Cha Yong CHoi, Hei Chan Lee, Jae Kyung Sohng, Keun Garp Ryu, Dae Joong Kim, Jong Seog Ahn, Beom Seok Kim, Hyun Cheol Oh, David H. Sherman, Yeo Joon Yoon "Structural Diversification of Macrolactones by Substrate-Flexible Cytochrome P450 Monooxygenases," **Advanced Synthesis and Catalysis,** 2005, 347, 1369-1378.
4. Sang Kil Lee, Je Won Park, Ji Won Kim, Won Seok Jung, Sung Ryel Park, Cha Yong Choi, Eung Soo Kim, Beom Seok Kim, Jong Seog Ahn, David H. Sherman, and Yeo Joon yoon, "Neopikromycin and Novapikromycin from the Pikromycin Biosynthetic Pathway of Streptomyces venezuelae," **Journal of Natural Products**, 2006, 69, 847-849.
5. Sang Kil Lee, Jay Sung Joong Hong, Cha Yong Choi, Jong Seog Ahn, Yeo Joon Yoon, "Enhanced production of hydroxyled macrolides from the pikromycin pathway of Streptomyces venezuelae," **Enzyme and Microbial Technology,** 2006, 39(4), 778-782.
6. Jung Won Seok, Sang Kil Lee, Jay Sung Joong Hong, Sung Ryeol Park, Soon Jeong Jeong, A Reum Han, Jae Kyung Sohng, Byung Gee Kim, Cha Yong Choi, David H. Sherman, and Yeo Joon Yoon, "Heterologous Expression of tylosin polyketide synthase and production of a hybrid bioactive macrolide in Streptomyces venezuelae," **Applied Microbiology and Biotechnology,** 2006, 72, 763-769.
7. Jay Sung Joong Hong, Su Jin Park, Niranjan Parajuli, Sung Ryul Park, Hwa Soo Koh, Won Seok Jung, Cha Yong Choi, Yeo Joon Yoon, "Functional analysis of DesVIII homologues involved in glycosylation of macrolide antibiotics by interspecies complementation," **Gene,** 2006, In Press.