

# Biosynthesis of dTDP-6-deoxy- $\blacklozenge$ -D-allose, biochemical characterization of dTDP-4-keto-6-deoxyglucose reductase (GerK1) from *Streptomyces* sp. KCTC 0041BP

Ta Thi Thu Thuy<sup>1</sup>, Kwangkyoung Liou<sup>1</sup>, Tae Jin Oh<sup>1</sup>,  
Doo Hyun Nam<sup>2</sup>, Jin Cheol Yoo<sup>3</sup> and Jae Kyung Sohng<sup>1\*</sup>

<sup>1</sup>*Institute of Biomolecule Reconstruction (iBR), SunMoon University, #100, Kalsan-ri, Tangjeong-myeon, Asansi, Chungnam 336-708, Korea.* <sup>2</sup>*Department of Pharmacy, Yeungnam University, Kyungbuk, 712-749, Korea.* <sup>3</sup>*Department of Pharmacy, Chosun University, Kwangju, 501-759, Korea*

dTDP-6-deoxy-D-allose, an unusual deoxysugar, has been identified as an intermediate in the mycinose biosynthetic pathway of several macrolide antibiotics. In order to characterize the biosynthesis of this deoxysugar, we have cloned and heterologously overexpressed *gerK1* in *E. coli* BL21 (DE3) cells. This gene encodes for a protein with the putative function of a dTDP-4-keto-6-deoxyglucose reductase, which appears to be involved in the dihydrochalcomycin (GERI-155) biosynthesis evidenced by *Streptomyces* sp KCTC 0041BP. Our results revealed that GerK1 exhibited a specific reductive effect on the 4-keto carbon of dTDP-4-keto-6-deoxyglucose, with the hydroxyl group in an axial configuration at the C3 position only. The enzyme catalyzed the conversion of dTDP-4-keto-6-deoxyglucose to dTDP-6-deoxy- $\blacklozenge$ -D-allose, according to the results of an *in vitro* coupled enzyme assay, in the presence of GerF (dTDP-4-keto-6-deoxyglucose 3-epimerase). The product was isolated, and its stereochemistry was determined via NMR analysis.

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