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Computational System for the Analysis of Post-PKS Glycosylation Step

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Polyketides are the components of a vast variety of microbial and plant secondary metabolites, including biologically active compounds such as antibiotics. The structural variety of polyketides is given by the options in choice of starter/extending units, number of condensations, and degree of subsequent reductive cycles, all of which being conferred by PKS and closely related enzymes. In addition, subsequent post-PKS tailoring steps provide the final product with further structural variations. In this context, we developed the computational system for the analysis of post-PKS glycosylation step. Our system carries the prediction and drawing of deoxysugar biosynthesis unit pathway, SBPD and GTDB searches, GT-encoding gene clustering, and back-office management. The deduced sugar biosynthesis unit pathway can allow us to determine and design the appropriate sugar that can be a substrate for glycosyltransferases and be employed in glycosylation of polyketides. The practical use of analysis result can provide researchers with the opportunity to conduct metabolic engineering of deoxysugar genes in collaboration with experimental work. The GT-encoding gene clustering and mapping may give clue to glycosyltransferase genes, each of which can be assigned to a specificGT reaction in biosyntesis. They usually possess regional and mechanistic specificites: α-(1,3)-glycosylation for UrdGT1c; β-(1,4)-glycosylation for UrdGT1b. A series of their chimeric GT genes can be constructed, and expressed in a mutants lacking corresponding glycosyltransfer activity to find the region determining the substrate specificities. Metabolic engineering to the region, including DNA shuffling, gives rise to the novel GT specificities leading to unnatural derivatives. Our system may be useful for experiment researchers to rationally design a novel polyketide and derivatives with biological activities.

Key words: Polyketide, Post-PKS tailoring steps, Glycosyltransferase, SBPD, GTDB

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Purification and Characterization of *Proteus mirabilis* Transcriptional Regulator, PMTR

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The *Proteus mirabilis* protein (PMTR) is a member of the MerR family by sequence alignments. Since the discovery of PMTR, an endogenous zinc-responsive *E. coli* protein, ZntR, has been identified. The *E. coli* ZntR is also a MerR family member and is activated by Cd(II), Pb(II), and Zn(II). Another chromosomal *E. coli* MerR-like protein has been characterized recently, the copper and silver-responsive CueR transcription regulator. Both ZntR and CueR regulate systems that confer resistance to the metals these regulators bind.

To analyze the DNA and metal binding properties of PMTR and its relationship to other metal binding MerR family members, Electrophoretic mobility shift assays (EMSA), footprinting experiments, and equilibrium binding assays, using fluorescence polarization, were done.