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*NUS Synthetic Biology for Clinical and Technological Innovation* (NUS SynCTI)
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**Professor Kim Byung Gee**
Department of Chemical and Biological Engineering
Seoul National University


## GT(Sialyltransferase and fucosyltransferase)mutagenesis and Metabolic Engineering for the synthesis of 3'/6'-SL and 2'/3-FL from human Milk

Oligosachharides in human milk(HMOs) have various useful biological functions for infant and applications in industry. Among them, sialyllactose(3'/6'-SL) and fucosyllactose(2'/3-FL) draw our atttention. To produce these oligosaccharides in large quantity, economic supply of CMP-Neu5Ac, highly active sialyltransferase(ST) and fucosyltransferase(FT) are keys to the success of the process development. Most sialylated oligosaccharides consist of Neu5Ac attached to galactose by an α2,3- or α2,6- linkage. In this research, α2,3-ST and α2,6-ST were engineered by hybrid approach to improve production of siayllactose. Hybrid approach is combined with rational design and directed evolution. This method can reduce library size by selecting target region such as substrate binding pocket and functional residues based on alanine scanning and computational mutation analysis. Saturation mutagenesis was done for selected residues to find best hits.

First, multifunctional α2,3 ST from Pasteurella multocida was engineered by this approach. We selected non-conserved residues located in substrates binding site by alignment with STs in GT80 family. And we applied alanine scanning for the selected residues. Mutants which show neutral activity were selected for saturation mutagenesis, and a single mutant interacting with lactose showed 168% of increased specific activity. Also, specific activity of a combination mutant was increased 200% compared to wild-type. In addition, α2,6 side reaction was reduced significantly for R313 mutants.

Second, α2,6 ST from Photobacterium damselae showed low activity and protein expression level. Therfore, it was engineered to increase catalytic activity. Substrate binding sites were predicted through homology modelling and functional residues were selected by the same method. Several mutants which show higher activity than wild type were screened by color assay method. Among them, single mutant interacting with CMP showed 4-5 times higher activity than wild type. Thus, α2,3- and α2,6-ST mutants obtained by hybrid approach will be an efficient tool for the improvement of production of sialyllactose.

In addition, Fucosyltransferases(FTs) are used for the synthesis of fucosyllactose(2'/3-FL) and sialyl-LewisX derivatives, and the same hybrid approach was undertaken. The major problems of FTs were their inclusion body formation and low level of soluble expression. How we have overcome this problem will be discussed. In the case of FL biosynthesis, rather than in vitro enzyme reaction, in vivo cell biocatalysis looks more promising. In result, metabolic engineering combined with protein engineering of FT becomes an essential approach to tackle the issues and problems for the biosysthesis of 2'/3-FL.


**BYUNG-GEE KIM** a,b, YUN-HEE CHOIa, BUM-SEOK PARK b
a Interdisciplinary Program for Biochemical Engineering and Biotechnology, Institute of Molecular Biology and Genetics, Seoul National University,151-742, Seoul, South Korea
b School of Chemical and Biological Engineering, Seoul National University, 151-742, Seoul, South Korea


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