Design of an β-L-transfucosidase for the synthesis of fucosylated HMOs

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Abstract:
Human milk oligosaccharides (HMOs) are recognized as benefiting breast-fed infants in multiple ways. As a result, there is growing interest in the synthesis of HMOs mimicking their natural diversity. Most HMOs are fucosylated oligosaccharides. β-L-Fucosidases catalyze the hydrolysis of β-L-fucose from the non-reducing end of a glucan. They fall into the glycoside hydrolase GH29 and GH95 families. The GH29 family fucosidases display a classic retaining mechanism and are good candidates for transfucosidase activity. We recently demonstrated that the β-L-fucosidase from Thermotoga maritima (TmβFuc) from the GH29 family can be evolved into an efficient transfucosidase by directed evolution (Osanjo et al. 2007). In this work, we developed semi-rational approaches to design an β-L-transfucosidase starting with the β-L-fucosidase from commensal bacteria Bifidobacterium longum subsp. infantis (BiAfcB, Blon_2336). Efficient fucosylation was obtained with enzyme mutants (L321P-BiAfcB and F34I/L321P-BiAfcB) enabling in vitro synthesis of lactodifucotetraose, lacto-N-fucopentaose II, lacto-N-fucopentaose III and lacto-N-difucohexaose I. The enzymes also generated more complex HMOs like fucosylated para-lacto-N-neohexaose (F-p-LNnH) and mono- or difucosylated lacto-N-neohexaose (F-LNnH-I, F-LNnH-II and DF-LNnH). It is worth noting that mutation at these two positions did not result in a strong decrease in the overall activity of the enzyme, which makes these variants interesting candidates for large-scale transfucosylation reactions. For the first time, this work provides an efficient enzymatic method to synthesize the majority of fucosylated HMOs.

Biography:

Dora Molnar-Gabor is a Head of DSP and Analytics at Glycom A/S Supporting and supervising the analytical activities of the R&D Analytical Group Analytical support of Human Milk Oligosaccharides production Development and implementation of new analytical methods and techniques for identification and quantitation of HMOs.

Recent Publications:

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