Novel α-L-Fucosidases from a Soil Metagenome for Production of Fucosylated Human Milk Oligosaccharides

This paper describes the discovery of novel α-L-fucosidases and evaluation of their potential to catalyse the transglycosylation reaction leading to production of fucosylated human milk oligosaccharides. Seven novel α-L-fucosidase-encoding genes were identified by functional screening of a soil-derived metagenome library and expressed in E. coli as recombinant 6xHis-tagged proteins. All seven fucosidases belong to glycosyl hydrolase family 29 (GH 29). Six of the seven α-L-fucosidases were substrate-inhibited, moderately thermostable and most hydrolytically active in the pH range 6-7, when tested with para-nitrophenyl-α-L-fucopyranoside (pNP-Fuc) as the substrate. In contrast, one fucosidase (Mfuc6) exhibited a high pH optimum and an unusual sigmoidal kinetics towards pNP-Fuc substrate. When tested for trans-fucosylation activity using pNP-Fuc as donor, most of the enzymes were able to transfer fucose to pNP-Fuc (self-condensation) or to lactose. With the α-L-fucosidase from Thermotoga maritima and the metagenome-derived Mfuc5, different fucosyllactose variants including the principal fucosylated HMO 2'-fucosyllactose were synthesised in yields of up to ~6.4%. Mfuc5 was able to release fucose from xyloglucan and could also use it as a fucosyl-donor for synthesis of fucosyllactose. This is the first study describing the use of glycosyl hydrolases for the synthesis of genuine fucosylated human milk oligosaccharides.

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