Substrate specificity and transfucosylation activity of GH29 α-l-fucosidases for enzymatic production of human milk oligosaccharides

Abstract Human milk oligosaccharides (HMOs) constitute a unique family of bioactive lactose-based molecules present in human breast milk. HMOs are of major importance for infant health and development but also virtually absent from bovine milk used for infant formula. Among the HMOs, the fucosylated species are the most abundant. Transfucosylation catalysed by retaining α-l-fucosidases is a new route for manufacturing biomimetic HMOs. Seven α-l-fucosidases from glycosyl hydrolase family 29 were expressed, characterized in terms of substrate specificity and thermal stability, and shown to be able to catalyse transfucosylation. The α-l-1,3/4-fucosidase CpAfc2 from Clostridium perfringens efficiently catalysed the formation of the more complex human milk oligosaccharide structure lacto-N-fucopentaose II (LNFP II) using 3-fucosyllactose as fucosyl donor and lacto-N-tetraose as acceptor with a 39% yield. α-l-Fucosidases FgFCO1 from Fusarium graminearum and Mfuc5 from a soil metagenome were able to catalyse transfucosylation of lactose using citrus xyloglucan as fucosyl donor. FgFCO1 catalysed formation of 2′-fucosyllactose, whereas Mfuc5 catalysis mainly produced an unidentified, non-HMO fucosyllactose, reaching molar yields based on the donor substrate of 14% and 18%, respectively.