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Review

Biotechnological production of fucosylated human milk oligosaccharides: Prokaryotic fucosyltransferases and their use in biocatalytic cascades or whole cell conversion systems

Barbara Petschacher[a,b], Bernd Nidetzky[a,b,*]

[a] Graz University of Technology, Institute of Biotechnology and Biochemical Engineering, NAWI Graz, Petersgasse 12, A-8010 Graz, Austria
[b] Austrian Centre of Industrial Biotechnology, Petersgasse 14, A-8010 Graz, Austria

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ABSTRACT

Human milk oligosaccharides (HMOs) constitute a class of complex carbohydrates unique to mother's milk and are strongly correlated to the health benefits of breastfeeding in infants. HMOs are important as functional ingredients of advanced infant formula and have attracted broad interest for use in health-related human nutrition. About 50% of the HMOs structures contain L-fucosyl residues, which are introduced into nascent oligosaccharides by enzymatic transfer from GDP-L-fucose. To overcome limitation in the current availability of fucosylated HMOs, biotechnological approaches for their production have been developed. Functional expression of the fucosyltransferase(s) and effective supply of GDP-L-fucose, respectively, are both bottlenecks of the biocatalytic routes of synthesis. Strategies of *in vitro* and *in vivo* production of fucosylated HMOs are reviewed here. Besides metabolic engineering for enhanced HMO production in whole cells, the focus is on the characteristics and the heterologous overexpression of prokaryotic α1,2- and α1,3/4-fucosyltransferases. Up to 20 g/L of fucosylated HMOs were obtained in optimized production systems. Optimized expression enabled recovery of purified fucosyltransferases in a yield of up to 45 mg/L culture for α1,2-fucosyltransferases and of up to 200 mg protein/L culture for α1,3/4-fucosyltransferases.

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Abbreviations: CDW, cell dry weight; FucT, fucosyltransferase; Fkp, fucokinase/L-fucose-1-phosphate guanylyltransferase; FL, fucosyllactose; GST, glutathione S-transferase; HMO, human milk oligosaccharide; MBP, maltose binding protein.

* Corresponding author at: Graz University of Technology, Institute of Biotechnology and Biochemical Engineering, NAWI Graz, Petersgasse 12, A-8010 Graz, Austria.
E-mail address: bernd.nidetzky@tugraz.at (B. Nidetzky).

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1. Introduction

1.1. Beneficial health effects of human milk oligosaccharides

Breastfeeding is known to bring forth remarkable health benefits for infants (Bode, 2012). The four most abundant solids in breast milk are lactose (70 g/L), lipids (40 g/L), complex lactose based oligosaccharides (5–15 g/L) and proteins (8 g/L) (Zivkovic et al., 2011). During the last decades, the complex oligosaccharides, also called human milk oligosaccharides (HMOs), have become a main focus of research on the benefits of breastfeeding. While the HMOs pass the gut and small intestine in principle undigested and therefore do not directly feed the newborns, they foster their health in several other ways.

Human milk as the predominant diet of newborns is an important environmental determinant of the gut microbiota of each neonate (Walker and Iyengar, 2015). HMOs act in the colon as prebiotics (Yu et al., 2013). Special health-beneficial microorganisms, such as *Bifidobacterium longum* subsp. *infantis* (Sela and Mills, 2010) can metabolize the HMOs (or certain components thereof) and therefore thrive, while other unfavorable microorganisms cannot utilize the HMOs and therefore are repressed (Bode, 2015).

In addition to this prebiotic function, also called bifidogenic effect, HMOs also act as soluble decoys for pathogens. Numerous pathogens, including viruses, bacteria and protozoa target glycoconjugates on the epithelial cell surface for adhesion. The epithelial oligosaccharide structures are related to the ABO and Lewis blood group determinants on erythrocytes. While ABO and Lewis antigens are carbohydrate structures bound to proteins or lipids on the cell surface, the HMOs resemble the same oligosaccharide structures in soluble form. Pathogens attach to HMOs, instead of epithelial cells, and subsequently get washed out of the intestine. The protective action of HMOs has been shown for infections of *Campylobacter jejuni*, enteropathogenic *Escherichia coli* and *Entamoeba histolytica* (Bode, 2015). Recently, the anti-adhesive effect on pathogens has also been shown for human cell lines treated with the two HMO structures 2′-fucosyllactose (2′-FL) and 3′-fucosyllactose (3′-FL) (Weichert et al., 2013). Lewis antigen structures also constitute the terminal parts of lipopolysaccharides decorating bacterial cell surfaces. In human pathogens, such as *Helicobacter pylori,* these oligosaccharides contribute to cell adhesion and to evasion of the host's immune response, thereby enabling long-term infections (Appelmelk et al., 1998; Chmiela, 2014).

HMOs have also been found to exhibit direct bacteriostatic (Bode, 2012) and antifungal (Gonia et al., 2015) properties, in addition to their gastro-intestinal anti-adhesive effect. Besides their local effects in the intestinal tract, HMOs can also enter the systemic circulation. Small amounts of HMOs could be traced in plasma and urine (Goehring et al., 2014). It has been proposed that these HMOs are involved in the protection of neonates by altering the immune response and increasing the resistance against uropathogens.

HMOs comprise a broad variety of oligosaccharide structures. Approximately 50% of the HMOs are fucosylated. The exact composition of the HMOs is individual for each mother and referred to as the person's milk glycobiome. The impact of the milk glycobiome on the gut microbiota of the neonate and its beneficial effects on health and development of the baby have recently been summarized in several detailed reviews (*e.g.* Bode, 2015, 2012; Pacheco et al., 2015; Smilowitz et al., 2014; Newburg, 2013).

In addition to health benefits for infants, HMOs might also be effective in promoting the health of adults due to their prebiotic and anti-adhesive properties. Moreover, the fucosylated HMOs resemble Lewis antigen glycan structures, which play an important role in inflammation and tumor metastasis. Sialylated Lewis structures are expressed on leukocytes and aid their adhesion to inflammation sites. Tumor cells hijack these structures and utilize them for metastasis formation (Glavey et al., 2015). Fucosylated HMOs are therefore highly desired for fundamental investigations and therapeutic trials in inflammation and cancer research.

In summary, due to the various beneficial effects to human health, HMOs have high application relevance. Use cases of HMOs comprise their application as supplements for neonate formulas, prebiotics, anti-adhesives, defined O-antigen structures for the development of vaccines against pathogens, inflammation mediators or tumor markers and/or repressors. Within the structural class of HMOs, fucosylated oligosaccharides are of special interest. To enable their promising application, especially in neonate formulas, fucosylated HMOs need to be available in suitable (*i.e.*, tons/year) amounts at reasonable prices. As the extraction from human milk is not feasible and their abundance in animal milk is too low for isolation, efficient synthesis methods are necessary to obtain the necessary quantities.

1.2. HMO structures

More than 200 HMO structures have been identified thus far. However, HMOs are built from only five monosaccharide building blocks, *i.e.* D-galactose (Gal), D-glucose (Glc), *N*-acetyl-D-glucosamine (GlcNAc), L-fucose (Fuc) and the sialic acid derivative *N*-acetyl-neuraminic acid.

The reducing end of HMOs is always built by lactose, which can be extended by several disaccharide units of two structure types, namely lacto-*N*-biose (Gal-β1,3-GlcNAc, type I) and lactosamine (Gal-β1,4-GlcNAc, type II, LacNAc). These disaccharides units are attached through β1,3 or β1,6 linkages to the galactosyl moiety of lactose (Fig. 1 and Table 1). Lactose or the peripheral Gal-β1,3-GlcNAc units of the HMO can be fucosylated or sialylated. Fucosyl residues are attached to Gal, GlcNAc and Glc through an α-glycosidic linkage. The sites of fucosylation vary between position 2, 3, or 4.

The content of fucosylated HMOs in human breast milk depends on the activity of two gene loci. The Se locus codes for an α1,2-fucosyltransferase (α1,2-FucT) and the Le locus codes for an α1,3/4-fucosyltranferase (α1,3/4-FucT) (Fig. 1). So called secretor women with an active Se locus express a functional α1,2-fucosyltransferase, their milk contains high amounts of α1,2-fucosylated HMOs, such as 2′-FL or lacto-*N*-fucopentaose I (H-type I antigen structure). Upon additional expression of an active α1,3/4-fucosyltransferase from the Le (Lewis) locus difucosylated structures with α1,3- or α1,4-linked fucosyl moieties appear. Lactodifucotetraose and lacto-*N*-difucohexaose I (Le^b-antigen structure) are the prominent products formed. If only the Le locus is active, structures like lacto-*N*-fucopentaose II (Le^a-antigen structure) and III (Le^x-antigen structure) and 3′-FL are detected in human milk. The most common phenotype, present in 70% of the