WEP Warwick Evans Publishing

International Journal of Public Health and Medical Research

ISSN: 3006-7707 (Print), ISSN: 3006-3752 (Online) | Volume 2, Number 1, Year 2024
DOI: https://doi.org/10.62051/ijphmr.v2n1.22
Journal homepage: https://wepub.org/index.php/IJPHMR/index

Research Progress on the Synthesis Methods of Human Milk Oligosaccharides

Boxin Qiao, Lei Liu, Jinlei Rong, Enxu Wang *

Bloomage Biotech (Tianjin) Technology Co., LTD, Tianjin 300000, China
*Corresponding Author: wangenxu@bloomagebiotech.com

ABSTRACT

Human milk oligosaccharides are the third largest solid component in breast milk and have functions such as regulating the intestinal flora, inhibiting the growth of pathogens, influencing the immune response of infants, and promoting brain development. Currently, the synthesis methods of human milk oligosaccharides include chemical synthesis, enzymatic synthesis and microbial fermentation. Chemical synthesis has low efficiency and doubts about product safety; enzymatic synthesis has a high conversion rate, but the substrate cost is high and the enzyme activity is unstable. Microbial fermentation can use Escherichia coli, yeast, Bacillus subtilis and Corynebacterium glutamicum as chassis strains, but it is not possible to determine the pros and cons of the research on chassis strains. At present, many kinds of human milk oligosaccharides have been approved by many countries as food additives or new raw materials and have application potential in many fields.

KEYWORDS

Human milk oligosaccharides; Chemical synthesis; Enzymatic synthesis; Microbial fermentation

1. INTRODUCTION

Human milk oligosaccharides (HMOs) are the third largest solid component in breast milk, which is formed by 3-6 sugar groups [1]. At present, more than 200 kinds of oligosaccharides have been determined [2], and they can usually be divided into acidic human milk oligosaccharides and neutral human milk oligosaccharides. Acidic human milk oligosaccharides contain sialic acid, such as 3′-sialyllactose (3′-SL) and 6′-sialyllactose (6′-SL). Neutral human milk oligosaccharides can be divided into fucosylated human milk oligosaccharides such as 2′-fucosyllactose (2′-FL), 3′-fucosyllactose (3′-FL), and difucosyllactose (DFL), and non-fucosylated human milk oligosaccharides such as lacto-N-tetraose (LNT) and lacto-N-neotetraose (LNnT) [3-5].

2. THE EFFICACY OF HUMAN MILK OLIGOSACCHARIDES

Human milk oligosaccharides can regulate the intestinal flora, enhance the intestinal barrier, promote the proliferation of probiotics and inhibit the growth of harmful bacteria [6-9]. Sialic acid oligosaccharides can inhibit the proliferation of intestinal epithelial cells and induce cell differentiation by binding to receptor of the epidermal growth factor (EGF), which can promote the maturation of intestinal [10-11]. Some human milk oligosaccharides, which contains the structure that is similar to the glycan receptor on the cell surface, can reduce the infection of intestinal epithelial cells by pathogens [12], such as LNT and LNnT can inhibit the adhesion of Streptococcus pneumoniae to epithelial cells [13]. HMOs can also regulate the expression of intestinal epithelial cells and indirectly affect the immune response of infants [14], such as 2′-FL inhibits the

inflammatory response by down-regulating the expression of lipopolysaccharide receptors on the surface of intestinal epithelial cells [15]. The sialic acid group existing in the structure of acidic human milk oligosaccharide can promote brain development by providing N-acetylneuraminic acid and gangliosides for infants during the growth stage [16-17]. In addition, the research demonstrates that 2′-FL can enhance cognitive ability, improve learning ability and enhance memory [18].

3. THE SYNTHESIS METHODS OF HUMAN MILK OLIGOSACCHARIDES

3.1. Chemical Synthesis Method

Chemical reactions, which are rapidly and efficiently, can obtain the products directly, due to the intermediate products do not require purification. Murata et al [19] used LNB-β-ρNP and lactose as the donor and receptor respectively to synthesize LNT by the transglycosylation reaction of lactoyl-N-diglycosidase, and when the reaction time was 3 hours, the yield was approximately 3.7%. Miermont et al. [20] used monosaccharides and lactose as the donor and receptor respectively to synthesize LNnT by assembling and modifying the monosaccharides by glycosylation reaction, with a yield of 40-60%. The overall synthesis efficiency of human milk oligosaccharides by chemical methods is low. In addition,

It needs multiple organic reagents during the synthesis process of human milk oligosaccharides that they are a kind of food-grade raw material, which exists the concerns about the safety of the product.

3.2. Enzymatic Synthesis Method

Enzymatic synthesis is a process to synthesize the final product through multi-step reactions using enzymes which are selected or modified from different microbial sources and different substrates with suitable conditions. Johnson et al. introduced the genes encoding β-1,3-acetylglucosamine aminotransferase (lgtA) and galactosyltransferase (lgtB) into Escherichia coli for expression to synthesize LNnT through a two-step method: the first step was to synthesize the intermediate product LNTII that was catalyzed by β-1,3-acetylglucosamine aminotransferase (lgtA) by using UDP-acetylglucosamine as the substrate and lactose as the receptor; the second step was to synthesize the final product LNnT that was catalyzed by galactosyltransferase (lgtB) by using UDP-galactose as the substrate and LNTII as the receptor; and the yield of the entire process was approximately 85% [21]. Wehmeier et al. prepared 2'-FL through two steps: the first step was to synthesize the intermediate product GDP-L-fucose that was catalyzed by GDP-D-mannose-4,6-dehydratase (Gmd) and GDP-L-fucose synthase (WcaG) by using GDP-D-mannose as the substrate; the second step was to synthesize the end product 2'-FL that was catalyzed by the glycosylation of α-1,2-fucosyltransferase by using GDP-L-fucose as the substrate and lactose as the receptor; and the yield of the entire process was approximately 65% [22]. The advantages of Enzymatic Synthesis are that is relatively simple for the reaction components and that is high for the conversion rate, however, it is the restricting factors for large-scale production to cost more on substrates and to maintain enzyme activity consistently stable.

3.3. Microbial Fermentation Method

The microbial fermentation method can utilize synthetic biology approaches to modify the metabolic pathways that exist within the microbial cells themselves or construct a set of metabolic pathways from scratch within the microbial cells for the production of target products. Currently, some engineered bacteria such as Escherichia coli, yeast, Bacillus subtilis, and Corynebacterium glutamicum have been modified for the production of human milk oligosaccharides by using synthetic biology and metabolic engineering techniques.

3.3.1. Escherichia coli for HMOs Production

Escherichia coli is a well-studied model microorganism. Compared with other microorganisms, it is studied more thoroughly on the genetic background and gene editing technology that leads to that is easier to edit and express genes. Parschat et al. [23] integrated the genes of the key pathway enzymes and the fucosyltransferase on the genome of the Escherichia coli to construct the de novo synthesis pathway that was used to produce 2′-FL in the process of fed-batch fermentation that leaded to a yield of 64 g/L by consuming sucrose that could synthesize the substrate GDP-L-fucose while providing energy and lactose with the catalysis of fucosyltransferase. Zhang et al. [24] imported the genes of the key enzymes that were used to synthetize the 5'-monophosphate adenosine nucleoside - disodium sialic acid (CMP-Neu5Ac) and the α-2,3-sialyltransferase (α-2,3-SiaT) into the Escherichia coli that was used to produce 3′-SL in the process of fed-batch fermentation that leaded to a yield of 23.1 g/L by consuming glycerol that could synthesize the substrate CMP-Neu5Ac while providing energy and lactose with the catalysis of α-2,3-SiaT. Hu et al. [25] screened 12 key genes that could affects the synthesis of LNnT by double-plasmid system in the Escherichia coli, and optimized the copy number of the key genes in a modular manner to affect the expression of key enzymes that could improve the yield that could reach 13.25 g/L by fed-batch fermentation of the product.

3.3.2. Yeast for HMOs Production

Yeast is a eukaryote and most of them have high safety, such as Saccharomyces cerevisiae. And yeast is often used as an engineered bacterium for industrial production, because it is convenient to achieve high-density cultivation due to the rapid reproduction of yeast and is less likely to form inclusion bodies during the cultivation process. Hollands et al. [26] integrated the gene of fucosyltransferase FucT2 into the Saccharomyces cerevisiae and Yarrowia lipolytica to constructe the de novo synthesis pathway of 2′-FL, meanwhile, the gene of Lac12 (derived from Kluyveromyces lactis) and the gene of CDT2 (derived from Neurospora crassa), which were screened to enhance the transport capacity of lactose and 2′-FL, were integrated respectively into the above two yeasts that were used to product 2′-FL that was obtained the yield that could reach 15 g/L and 24 g/L respectively by fed-batch fermentation. Lee et al. [27] imported the genes of the enzymes of Gmd, WcaG, and WbgL to construct the de novo synthesis pathway of 2′-FL and the gene of the lactose transporter (Lac12) that could accelerate the transport of lactose into the Saccharomyces cerevisiae that could utilize xylose efficiently, there was 25.5 g/L 2'-FL could be produced with with xylose as the carbon source and lactose as the substrate in the fed-batch fermentation.

3.3.3. Bacillus subtilis for HMOs Production

Bacillus subtilis is a Gram-positive bacterium and generally regarded as safe (GRAS). It proliferates faster and has a high ability to secrete and produce recombinant proteins, which has important industrial application value in metabolic engineering and synthetic biology. Ji et al. [28] constructed the de novo synthesis pathway of 2′-FL in Bacillus subtilis and improved the utilization rate of lactose by knocking out the β-galactosidase that could degrade lactose and importing the gene of lacY that could enhance the transport capacity of lactose, there was 31.2 g/L of 2′-FL could be produced with xylose and glycerol as carbon sources and lactose as the substrate in the fed-batch fermentation. Dong et al. [29] imported the genes of β-1,3-acetylglucosamine aminotransferase (lgtA) and β-1,4-galactosyltransferase (LgtB) into Bacillus subtilis which carried the gene of β-galactoside permease(LacY) that could improve the utilization rate of lactose to construct the LNnT synthesis pathway, there was 4.52 g/L of LNnT could be produced with glucose as carbon sources and lactose as the substrate in the fed-batch fermentation when the supply of the two precursors UDP-GlcNAc and UDP-Gal was balanced by optimizing the expression levels of lgtA and LgtB. Zhang et al. [30] constructed the de novo synthesis pathway of 2′-FL in Bacillus subtilis to obtain the final strain BS21 by knocking out the gene of β-galactosidase that could reduce the consumption of lactose, over expressing the gene of the ndk that could promote the conversion of GMP to GTP to enhance energy supply, and optimizing the type of promoter of the pathway enzymes. It was 88.3 g/L of 2′-FL to be

produced by using a 3L fermenter for the verification of the strain, which was the highest yield of 2′-FL prepared with Bacillus subtilis as the chassis strain at present.

3.3.4. Corynebacterium glutamicum for HMOs Production

Corynebacterium glutamicum is a kind of Gram-positive bacteria and generally regarded as safe (GRAS) [31] in microbiology, which has endogenous phosphomannomutase gene (manB) and GTP-mannose-1-phosphate guanyltransferase gene (manC) [32]. Chin et al [33]. constructed the de novo synthesis pathway that introduced the gmd and wcaG genes which were derived from Escherichia coli into Corynebacterium glutamicum to obtain 86.2 mg/L GDP-L-fucose by optimizing the expression levels of manB, manC, gmd and wcaG genes. It is successful to construct the metabolic pathway of GDP-L-fucose that is an important precursor substance for the synthesis of 2′-FL and 3′-FL, which provides the possibility to synthesize 2′-FL and 3′-FL. Seo et al. [34] constructed the de novo synthesis pathway of 2′-FL that could be produced 8.1 g/L in the fed-batch with glucose as the carbon source by introducing the genes of GDP-mannose dehydratase (Gmd), GDP-L-fucose synthase (WcaG), lactose permease (Lac Y) and α-1,2-fucosyltransferase into Corynebacterium glutamicum

Currently, Escherichia coli which has simple structure and is researched more mature on genetic background and gene editing technology is the most of the chassis strains that is usually can get a high yield for the production of HMOs. Due to Escherichia coli has the disadvantages that can be infected phage easily and can produce endotoxins, some of the other GRAS microbial chassis strains which is used to produce HMOs, such as Saccharomyces cerevisiae, Yarrowia lipolytica, Bacillus subtilis and Corynebacterium glutamicum, etc, have fewer available genetic elements and dynamic regulation tools [35], which will generate significant challenges to construct strain and improve yield.

4. CONCLUSION

At present, various types of human milk oligosaccharides have been approved by different countries or regions that include FDA, EFSA or FSANZ, etc, as food additives or new food raw materials to be added to foods. In 2023, the National Health Commission of China approved two types of human milk oligosaccharides as new varieties of food nutrition fortifiers, allowing them to be used in formulated milk powder, infant formula foods, and infant formula foods for special medical purposes. It is reported that the global biosynthetic HMO market has achieved sales of 459 million dollars in 2022 that has more than 87% in the North American and European and is expected to reach 2.153 billion dollars in 2029 that is expected to have the fastest growth which is up to 46.37% of CAGR in the Asia-Pacific region, which has a compound annual growth rate of 24.55%. It is a large market and developing foreground for HMO on the areas such as infant formula milk powder, health supplements, health care, animal health, and personal care, etc, and with the development of technology, the market space will be continued to expand.

AUTHOR CONTRIBUTION STATEMENT

All authors listed have significantly contributed to the development and the writing of this article.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGMENTS

This study received the support of Bloomage Biotech (Tianjin) Technology Co., LTD.

REFERENCES

[1] Zhu Y, Wan L, Li W, et al. Recent advances on 2′-fucosyllactose: physiological properties, applications, and production approaches [J]. CriticalReviews in Food Science and Nutrition, 2022, 62(8): 2083-2092.

[2] Bode L. Human milk oligosaccharides: Every baby needs a sugar mama [J]. Glycobiology, 2012, 22(9): 1147-62.

[3] Grollman A P, Hall C W, Ginsburg V. Biosynthesis of Fucosyllactose and Other Oligosaccharides Found in Milk [J]. Journal of Biological Chemistry, 1965, 240(3): 975-981.

[4] Sprenger G A, Baumgartner F, Albermann C. Production of human milk oligosaccharides by enzymatic and whole-cell microbial biotransformations [J]. Journal of Biotechnology, 2017, 258:79-91.

[5] Anderson A, Donald A S R. Improved method for the isolation of 2′-fucosyllactose from human milk [J]. Journal of Chromatography A, 1981, 211(1): 170-174.

[6] Nolan L S, Rimer J M, Good M. The role of human milk oligosaccharides and probiotics on the neonatal microbiome and risk of necrotizing enterocolitis: a narrative review. Nutrients, 2020, 12(10): 3052.

[7] Iribarren C, Magnusson M K, Vigsnaes L K, et al. The effects of human milk oligosaccharides on gut microbiota, metabolite profiles and host mucosal response in patients with irritable bowel syndrome [J]. Nutrients, 2021, 13(11): 3836.

[8] Spicer S K, Gaddy J A, Townsend S D. Recent advances on human milk oligosaccharide antimicrobial activity [J]. Current Opinion in Chemical Biology, 2022, 71: 102-202.

[9] Lin A E, Autran C A, Szyszka A, et al. Human milk oligosaccharides inhibit growth of group B Streptococcus [J]. Journal of Biological Chemistry, 2017, 292(27): 11243-11249.

[10] Donovan S M. Human milk oligosaccharides-the plot thickens [J]. The British Journal of Nutrition, 2009, 101(9): 1267-1269.

[11] Kawashima N, Yoon S J, Itoh K, et al. Tyrosine kinase activity of epidermal growth factor receptor is regulated by GM3 binding through carbohydrate to carbohydrate interactions [J]. The Journal of Biological Chemistry, 2009, 284(10): 6147-6155.

[12] Bode L. The functional biology of human milk oligosaccharides [J]. Early Human Development, 2015, 91(11): 619-622.

[13] Andersson B, Porras O, Hanson L A, et al. Inhibition of attachment of Streptococcus pneumoniae and Haemophilus influenzae by human milk and receptor oligosaccharides [J]. The Journal of infectious diseases, 1986, 153(2): 232-237.

[14] Hanson L A. Breast-feeding and immune function [J]. Proceedings of the Nutrition Society, 2007, 66(3): 384-396.

[15] He Y, Liu S, Kling DE et al. The human milk oligosaccharide 2′-fucosyllactose modulates CD14 expression in human enterocytes, thereby attenuating LPS-induced inflammation [J]. Gut, 2016, 65: 33-46.

[16] Wang B. Sialic acid is an essential nutrient for brain development and cognition [J]. Annual Review of Nutrition, 2009, 29(1): 177-222.

[17] Wang B, Brand - Miller J. The role and potential of sialic acid in human nutrition [J]. European journal of clinical nutrition, 2003, 57(11): 1351-1369.

[18] Marriage B j, Buck R H, Goehring K C, et al. Infants fed a lower calorie formula with 2′-FL show growth and 2′-FL uptake like breast-fed infants [J]. Journal of Pediatric Gastroenterology and Nutrition, 2015, 61(6): 649-658.

[19] Murata T, Inukai T, Suzuki M, et al. Facile enzymatic conversion of lactose into lacto-N-tetraose and lacto-N-neotetraose [J]. Glycoconjugate Journal, 1999, 16: 189-195.

[20] Miermont A., Zeng Y., Jing Y., et al. Syntheses of LewisX and dimeric LewisX: Construction of branched oligosaccharides by a combination of preactivation and reactivity based chemoselective one-pot glycosylations [J]. Journal of Organic Chemistry, 2007, 72 (23): 8958-8961.

[21] Karl F Johnson Synthesis of oligosaccharides by bacterial enzymes [J]. Glycoconjugate Journal, 1999, 16 (2): 141-146.

[22] Wehmeier P U F. Synthesis of the milk oligosaccharide 2′-fucosyllactose using recombinant bacterial enzymes [J]. Carbohydrate Research, 2001, 334(2): 97-103.

[23] Parschat K, Scheriber S, Wartenberg D, et al. High-titer de novo biosynthesis of the predominant human milk oligosaccharide 2′-fucosyllactose from sucrose in Escherichia coil [J]. ACS Synth Biol, 2020, 9(10): 2784-2796.

[24] Zhang JM, Zhu YY, Zhang WL, Mu W. Efficient production of a functional human milk oligosaccharide 3'-sialyllactose in genetically engineered Escherichia coli [J]. ACS Synthetic Biology, 2022, 11(8): 2837-2845.

[25] Hu M, Li M, Li C, et al. High-level productivity of lacto-n-neotetraose in Escherichia coli by systematic metabolic engineering [J]. Journal of Agricultural and Food Chemistry, 2023, 71(9): 4051-4058.

[26] Hollands K, Baron C M, Gibson K J, et al. Engineering two species of yeast as cell factories for 2′-fucosyllactose [J]. Metabolic Engineering, 2019, 52: 232-242.

[27] Lee J W, Kwak S, Liu J J, et al. Enhanced 2'-fucosyllactose production by engineered Saccharomyces cerevisiae using xylose as a co-substrate [J]. Metablic Engineering, 2020, 62: 322-329.

[28] Ji M, Liu Y, Xie S, et al. De novo synthesis of 2'-fucosyllactose in engineered Bacillus subtilis ATCC 6051a [J]. Process Biochemistry, 2022, 120: 178-185.

[29] Dong X., Li N., Liu Z., et al. Modular pathway engineering of key precursor supply pathways for lacto-N-neotetraose production in Bacillus subtilis [J]. Biotechnology for Biofuels, 2019, 12 (1): 212.

[30] Zhang Q W, Liu Z M, Liu L, et al. Engineered bacillus subtilis for the de novo production of 2'-fucosyllactose [J]. Microbial Cell Factories, 2022, 21(1): 1-11.

[31] Lu M, Mosleh I, Abbaspourrad A. Engineered microbial routes for human milk oligosaccharides synthesis [J]. ACS Synthetic Biology, 2021, 10(5): 923-938.

[32] WENG Ruru, WEI Xinhui, LI Haozheng, et al. Progress in Microbial Synthesis of 2'-Fucosyllactose [J]. Food Science, 20221, 42(17): 248-254.

[33] Chin YW, Park JB, Park YC, et al. Metabolic engineering of Corynebacterium glutamicum to produce GDP-L-fucose from glucose and mannose. Bioprocess Biosyst Eng. 2013; 36(6):749-756.

[34] Seo J-H, Chin Y-W, Jo H-Y. Method of producing 2′-fucosyllactose using Corynebacterium glutamicum [P]. US Patent, 20180298389A1. 2018.

[35] Zhang Q W, Wu Y K, Gong M Y, et al. Production of proteins and commodity chemicals using engineered Bacillus subtilis platform strain. Essays in Biochemistry, 2021, 65(2): 173-185.