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1 **Functional properties, structural studies and chemo-enzymatic synthesis of**
2 **oligosaccharides**

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17

18 **Abstract:** Oligosaccharides offer beneficial effects on immune system and gut health, such
19 as anticancer activity, immunomodulatory activity, and complement activation. Functional
20 oligosaccharides are widely found in plants, algae, bacteria and higher fungi. Milk
21 oligosaccharides, especially human milk oligosaccharides, have considerable health
22 benefits, such as the growth-promotion of the beneficial bacterial flora in the intestines, and
23 developing resistance to bacterial and viral infections. Recent developments in high
24 performance liquid chromatography, mass spectrometry, nuclear magnetic resonance and
25 capillary electrophoresis techniques contribute to the analysis of the oligosaccharide
26 identification and mixture quantification. Synthesis of oligosaccharides is becoming
27 increasingly important to pharmaceutical industries, in which chemo-enzymatic synthesis
28 is considered as an effective method. This article gives a brief summary of structures,
29 accessible sources, physiological and chemical characteristics, and potential health benefits
30 of functional oligosaccharides.

31
32 **Keywords:** Oligosaccharides; Functional properties; Structural analysis; Milk
33 oligosaccharides; Chemo-enzymatic synthesis

36 1. Introduction

37 Personal health has become an ever-increasing important issue for consumers. Identification
38 and characterization of functional food components have advanced nutrition science.
39 Non-digestible dietary fibers and functional oligosaccharides are functional carbohydrates
40 with various benefits (Bland, Keshavarz, & Bucke, 2004). According to the IUPAC-IUBMB
41 Joint Commission on Biochemical Nomenclature, naturally occurring carbohydrates that
42 consist of 3–10 monosaccharide units, linear or branched, connected by α - and/or
43 β -glycosidic linkages, are defined as oligosaccharides (or glycans). However, the
44 physiological or rational chemical reasons for setting these limits remains unclear.
45 Carbohydrates, whose monosaccharide units are fructose, galactose, glucose and/or xylose,
46 are recognized as the main classes of functional oligosaccharides available at present or
47 under development (Mussatto & Mancilha, 2007) (Fig. 1). These molecules are well-known
48 as prebiotics, because they promote the growth of beneficial bacteria, particularly
49 *Bifidobacteria* species. These functional oligosaccharides have shown advantageous
50 physicochemical and physiological properties that contribute to the improvement of
51 consumer health. Thus, application of oligosaccharides as ingredients in functional foods has
52 great potential for improving the quality of foods in relation to consumers' health.

54 2. Health benefit of functional oligosaccharides

55 Functional oligosaccharides have been applied for many purposes, such as nutrients,
56 pharmaceuticals, feeds, cosmetics, immunostimulating agents and prebiotic compounds
57 (Patel & Goyal, 2011; Sako, Matsumoto, & Tanaka, 1999), which incorporate 13 classes of
58 commercially produced non-digestible oligosaccharides showing bifidogenic functions. In
59 addition, known functional oligosaccharides also include arabino-oligosaccharides,
60 arabinogalacto-oligosaccharides, arabinoxylo-oligosaccharides, galacturono-
61 oligosaccharides, rhamnogalacturonoligosaccharides, and human milk oligosaccharides
62 (HMOs) (Table 1). In particular, cyclodextrins produced from starch through enzymatic
63 conversion in nature is a family of macrocyclic oligosaccharides (Astray, Gonzalezbarreiro,
64 Mejuto, Rial-Otero, & Simal-Gandara, 2009; Radu, Parteni, & Ochiuz, 2016). Cyclodextrins
65 (α , β , and γ) are cyclic α -(1 \rightarrow 4)-glucans with degrees of polymerization of 6, 7, and 8
66 monosaccharide units, respectively. Macrocyclic carbohydrates have been widely applied as
67 building-blocks in supramolecular chemistry, drug carriers, molecular reactors, and artificial
68 receptors (Muthana, Yu, Cao, Cheng, & Chen, 2009). The use of functional oligosaccharides
69 improves the balance of the intestinal microflora and greatly decreases the gastrointestinal

70 infections (Xu, Chao, & Wan, 2009). Additionally, the consumption of functional
71 oligosaccharides can reduce the risk of lifestyle-related diseases, such as cardiovascular
72 disease, cancer, obesity and type 2 diabetes, which are related to obesity (Mussatto &
73 Mancilha, 2007) (Table 2). Thus, functional oligosaccharides are widely cited to be
74 important dietary fibers in nutritional advice for metabolic syndromes induced specific
75 disorders.

76

77 **3. Sources of functional oligosaccharides**

78 Plants and algae are the richest sources of functional oligosaccharides (Van Laere,
79 Hartemink, Bosveld, Schols, & Voragen, 2000) (Table 2). Depolymerization of suitable raw
80 materials or partial enzymatic hydrolysis of purified pectins can produce the pectic
81 oligosaccharides (Gullón, Gómez, Martínez-Sabajanes, Yáñez, Parajó, & Alonso, 2013).
82 Some typical feruloylated oligosaccharides could be prepared from plant sources, e.g.,
83 wheat, maize bran, sugarcane bagasse and rice (Qu & Sun, 2014). Particularly, marine
84 oligosaccharides have attracted attention in drug development (Zhao, Wu, Yang, Liu, &
85 Huang, 2015). Carrageenans, extracted from marine red algae, belong to an anion polymers
86 family and share a common backbone of alternating (1→3)-linked β -D-galactopyranose and
87 (1→4)-linked α -D-galactopyranose (Yao, Wu, Zhang, & Du, 2014). Carrageenans are
88 well-known for their valuable biological activities, mainly attributed to the presence of
89 sulphates (Kim & Rajapakse, 2005). Chitosan and its derivatives show potential in various
90 fields such as food, cosmetics, biomedicine and agriculture. Chitosan oligosaccharides have
91 low viscosity and high solubility in water, particularly at neutral pH. Recent studies have
92 focused on the health benefits of chitosan oligosaccharides, such as decreasing blood
93 cholesterol, controlling of high blood pressure, protecting from infections, and improving the
94 antitumor properties (Zou et al, 2015).

95

96 **4. Milk oligosaccharides**

97 Milk has evolved as a complete food for mammalian nourishment during infancy. Milk
98 oligosaccharides are the most relevant prebiotic components (Mills, Ross, Hill, & Stanton,
99 2011; Boehm & Stahl, 2007). The concentration of total oligosaccharides in the milk of most
100 mammals is much too low. Moreover, the major type of oligosaccharides in human milk,
101 fucosylated oligosaccharides, was not detected in mammalian milk (Mehra & Kelly, 2006).
102 The concentration of human milk oligosaccharides (HMOs) in mature human milk is 10–15
103 g/L, which is 100- to 1000-fold higher than that in bovine milk, and the content of HMOs

104 often exceeds the total amount of protein in mature human milk (Table 3). Complex
105 oligosaccharides, particularly unconjugated complex glycans, HMOs, make up a high
106 percentage of the total solids in human milk. Nearly 200 HMOs have been identified, among
107 which more than 80 have been fully characterized from a structural perspective. The
108 biological functions of HMOs are closely associated with their structural conformation
109 (Bode, 2015). Galactose, glucose, fucose, *N*-acetylglucosamine, and the sialic acid
110 derivative, *N*-acetyl-neuraminic acid are the five monosaccharide building blocks that can
111 constitute HMOs (Kobata, 2010). These glycans can be fucosylated and/or sialylated (Fig.
112 2). All HMOs carry lactose (Gal β 1-4Glc) at the reducing end, which can be elongated in a
113 β -1,3 or β -1,6-linkage by two different disaccharides, either type 1 carbohydrate structures
114 (containing Gal β 1-3GlcNAc units) or type 2 structures (containing Gal β 1-4GlcNAc units).
115 HMOs with more than 15 disaccharide units can form complex structural backbones and be
116 further modified by adding fructose and/or sialic acid. Studies have demonstrated that HMOs
117 can induce increased levels of bifidobacteria in the colonic flora of breast-fed infants,
118 accompanied by a great reduction in pathogenic potential bacteria, by the bifidogenic
119 activity of HMOs (Jin, Joo, Li, Choi, & Han, 2016). HMOs were shown to greatly affect the
120 composition of the gut microflora. The HMOs lacto-*N*-fucopentaose I could be selectively
121 utilized by *Bifidobacterium longum* subsp. *infantis*, but not *B. animalis* subsp. *lactis*, making
122 it a promising potential prebiotic (Zhao et al., 2016). HMOs protect from viral, bacterial, or
123 protozoan pathogens and affect fungal–host interactions (Hong, Ninonuevo, Lee, Lebrilla,
124 & Bode, 2009; Shoaf-Sweeney & Hutkins, 2009).

125

126 **5. Structural analysis of glycan oligosaccharides**

127 Because of the complexity and heterogeneity of oligosaccharides, characterization
128 technologies for oligosaccharides are not as advanced as the technologies for characterizing
129 nucleic acids and proteins. Moreover, oligosaccharides are particularly difficult to separate,
130 analyze and obtain detail structural information due to the coexisting isomeric structures and
131 multiple connectivity sites. Many techniques have been developed to elucidate
132 oligosaccharide structural characterization in order to understand their specific functions,
133 however there is no legal method for analyzing and quantifying oligosaccharides (Table 4).
134 The sensitive method of high-resolution mass spectrometry (HR-MS), which can provide a
135 good breadth of information, has become a main tool for oligosaccharide analysis (Bao,
136 Chen, & Newburg, 2013). Oligosaccharides fractionation attained by gel permeation
137 chromatography (GPC) followed by analysis of high-molecular mass fractions by

138 matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
139 (MALDI-TOF/MS) and electrospray ionization ion trap mass spectrometry (ESI-ITMS)
140 indicated that complex oligosaccharides have a larger mass range compared to previous
141 techniques (Hsu, Chang, & Franz, 2006). Although MALDI-MS has been used successfully
142 to characterize underivatized oligosaccharides, MALDI-TOF/MS and MALDI post-source
143 decay TOF/MS analysis are ten-fold more sensitive than MALDI-MS (Park, Yang, Kim, &
144 Kim, 2012). Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS)
145 can be used to analyze oligosaccharides directly or with analytical derivatization (Wang,
146 Chu, Zhao, He, & Guo, 2011). A combination of negative-ion electrospray tandem mass
147 spectrometry (ES-MS/MS), methylation analysis, and ^1H nuclear magnetic resonance
148 spectroscopy (^1H -NMR) has been applied to identify new oligosaccharides. ^{13}C - and
149 ^1H -NMR, together with ES-MS, have been applied to determine the structures of complex
150 sulfated oligosaccharides isolated from human milk (Balogh, Szarka, & Béni, 2015).
151 FT-ICR has been used to detect oligosaccharides recently (Lee, An, Lerno, German, &
152 Lebrilla, 2011). Additionally, the efficient method of nano-electrospray ionization mass
153 spectroscopy (nESI-MS) with quadrupole ion trap has been used to identify the position of
154 fucose, types of linkages, and differentiation of linear and branched structures of isomeric
155 oligosaccharides from a complex mixture of native underivatized neutral oligosaccharides
156 (Pfenninger, Karas, Finke, & Stahl, 2002). Detect interactions of proteins with glycans or
157 glycoconjugates by nESI-MS. The development of additional techniques may result in
158 structural characterization of isolated oligosaccharides. The structures of HMOs are quite
159 complex and novel techniques such as porous graphitic carbon (PGC) LC-MS are now
160 available to perform the separation and identification of most isomers (Ruhaak, Lebrilla,
161 Weimer, & Slupsky, 2013). Oligosaccharides studies will benefit from the application of the
162 most advanced analytical methods, such as high performance anion exchange
163 chromatography (HPAEC) with pulsed amperometric detection (PAD) or capillary
164 electrophoresis (CE), which can be used to measure samples at picomole and femtomole
165 levels, respectively (Monti, Cattaneo, Orlandi, & Curadi, 2015; Morales, Corzo, & Sanz,
166 2008).

167

168 **6. Chemo-enzymatic synthesis of bioactive oligosaccharides**

169 Since the nature cannot always provide enough amounts of such functional carbohydrates for
170 scientific research or clinical applications, development of new techniques to improve the
171 production of such carbohydrates has become a new challenge in glycoscience. The

172 development of automated methods can meet the demand for molecular tools for rapid
173 analysis of glycobiology. A few major advances in carbohydrate synthesis have been
174 observed in recent years (Bartolozzi & Seeberger, 2001). Current methods for obtaining
175 synthetic oligosaccharides are chemical synthesis or chemo-enzymatic synthesis (Muthana,
176 Yu, Cao, Cheng, & Chen, 2009). The chemical synthetic pathway is challenging due to the
177 numerous protection and deprotection steps; the large amounts of reagents and organic
178 solvents that are often toxic; and carrying out the reactions under harsh conditions. The
179 laborious chemical synthetic pathway frequently results in low yields. The chemo-enzymatic
180 method involves glycosyltransferases (GT) and glycosidases (GH), which are the enzymes
181 naturally involved in oligosaccharide synthesis in prokaryotes and eukaryotes (Yu & Chen,
182 2016). By exploiting these enzymes in the laboratory for the synthesis of oligosaccharides,
183 many of the challenges faced when using chemical synthesis could be overcome. Some
184 different complex oligosaccharides and derivatives with 3–11 monosaccharides units have
185 been reported by preparative-scale and improved large-scale productions (Table 5).

186 Chemo-enzymatic methods can be applied in the synthesis of virtually any complex
187 oligosaccharide (Hanson, Best, Bryan, & Wong, 2004). Enzymatic coupling has some
188 advantages over its chemical counterpart. The use of enzymes in the synthesis of
189 oligosaccharides has attracted growing interest as an alternative to chemical synthesis
190 (Koeller & Wong, 2001). Glycosyltransferase-catalyzed enzymatic and chemo-enzymatic
191 syntheses are widely considered to be effective ways for oligosaccharides production (Fig.
192 3). Enzymatic glycosylation occurs stereo- and regioselectively under mild conditions
193 without protecting group manipulation. Functional enzymes enable the large-scale synthesis
194 of difficult-to-produce saccharide linkages and complex molecules. An efficient one-pot
195 multienzyme fucosylation system used for the gram-scale synthesis of lacto-*N*-fucopentaose
196 I has been reported recently (Zhao et al., 2016). The development of one-pot
197 multienzyme-catalysed syntheses reduces the substrate costs for *in vitro* production of
198 fucosylated carbohydrates (Yu & Chen, 2016). Additionally, cost-effective large-scale
199 production of HMOs may be conducted using whole cell systems; thus *in vitro* synthesis
200 offers the unique advantage of flexibility. Moreover, small-scale enzymatic synthesis of
201 structurally complex HMOs, which cannot currently be produced in engineered cells, is an
202 invaluable tool for supporting studies on biological function and possible applications of
203 these oligosaccharide structures.

204 GT's and GH's are stereo- and regioselective, therefore circumventing the tedious
205 protection/deprotection steps. The reaction conditions are generally mild and can be carried

206 out in physiological buffers and temperatures eliminating the use of harsh conditions and
207 toxic chemicals. In addition, enzymes are highly efficient and have flexibility on the
208 substrates, which often result in great yields of oligosaccharide products. A disadvantage to
209 the chemo-enzymatic method is identifying active GT and GH using recombinant expression
210 systems and determining the enzyme's preferred substrate. Obtaining good expression levels
211 in recombinant expression systems can be challenging. For example, proteins expressed in
212 *Escherichia coli* systems may aggregate due to misfolding and many eukaryotic proteins
213 require post-translational modifications for activities. With the advent of whole genomic
214 sequences across species, putative GT's and GH's have been inferred by sequence homology
215 studies.

216

217 **7. Conclusions**

218 In summary, the functional oligosaccharides are associated with a variety of biological
219 processes such as resistance against the infection of bacteria and virus, antioxidant,
220 antimutagenicity, cancer metastasis inhibition, blood-clotting cascade and many other
221 pharmacological activities. However, the synergistic effect of a mixture of more structurally
222 oligosaccharides from the nature sources should also be investigated as, most likely, one
223 single will not provide the desired function. More efforts need to be applied for the
224 production of more complex oligosaccharides, especially the ones that are branched. The
225 large-scale production of oligosaccharides using multiple OPME systems or engineered *E.*
226 *coli* living-cell fermentation approaches would promote a new era for oligosaccharides
227 synthesis. The development of universal sequencing tools for oligosaccharides with
228 comparable speed and throughput still remains a challenge, and the most advanced
229 analytical techniques are promising to be useful tools. The identification, production and
230 commercialization of new functional oligosaccharides with enhanced bioactive properties
231 offer new research and business opportunities. They are good candidates for various
232 applications in food and pharmacological industry.

233

234 **Abbreviations**

IUPAC	International Union of Pure and Applied Chemistry
IUBMB	International Union of Biochemistry and Molecular Biology
CDs	Cyclodextrins
HMOs	Human milk oligosaccharides
GlcNAc	<i>N</i> -acetyl-glucosamine

Neu5Ac	<i>N</i> -acetyl-neuraminic acid
MS	Mass spectrometry
MALDI-TOF	Matrix-assisted laser desorption/ionization time-of-flight
GT	Glycosyltransferases
GH	Glycosidases

235

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240

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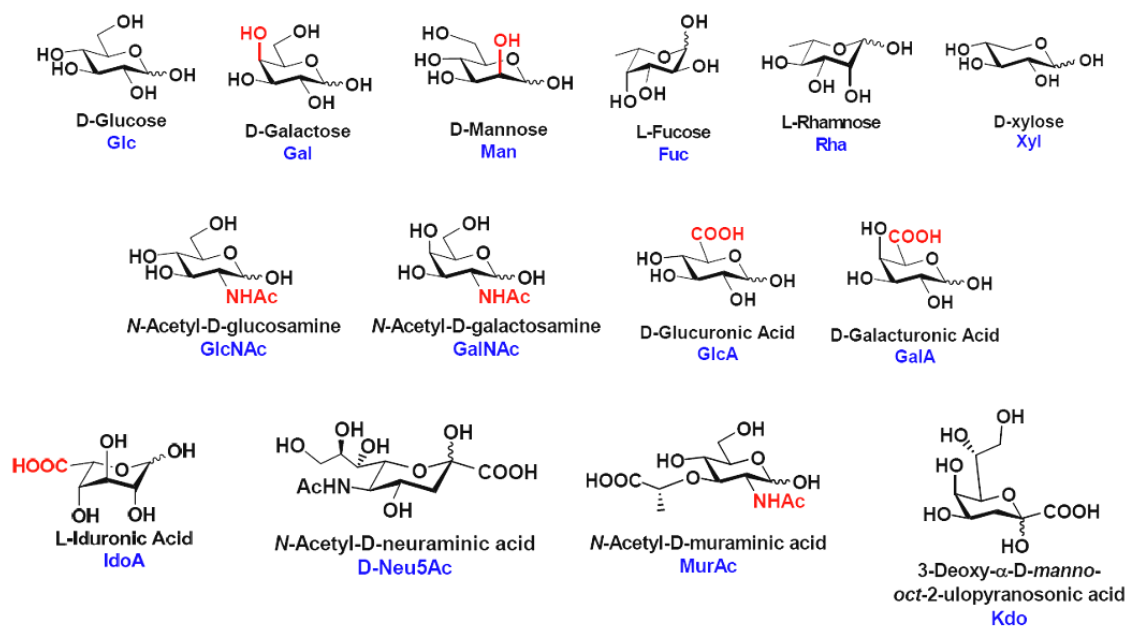
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Fig. 1 Common monosaccharides components of the functional oligosaccharides.

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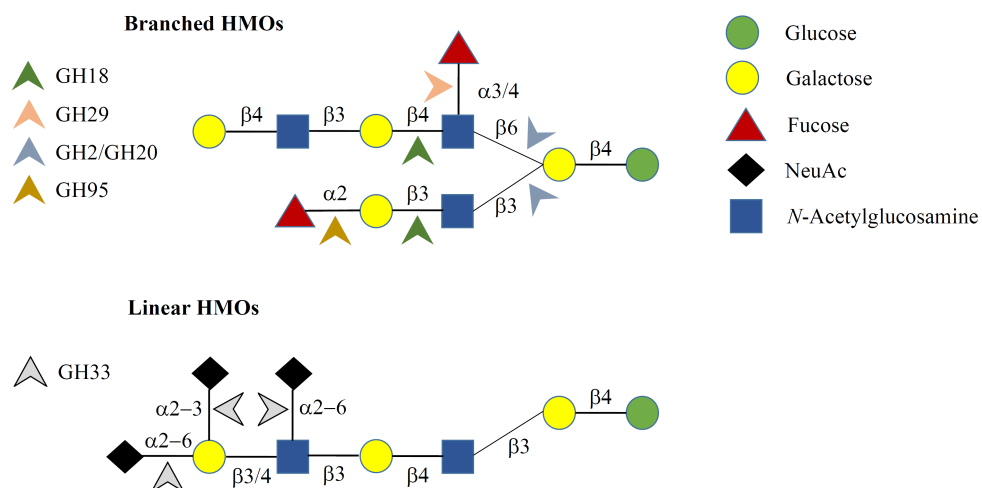
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612 **Fig. 2** Schematic of HMOs structures and putative HMOs active glycoside hydrolase families (GH) based on
 613 the work of Wu, Tao, German, Grimm, & Lebrilla (2010). GH2, α -galactosidase; GH18,
 614 endo- β -N-acetylglucosaminidase; GH20, β -hexosaminidase; GH29, α -1,3/4-fucosidase; GH33, sialidase;
 615 GH95, α -1,2-fucosidase.

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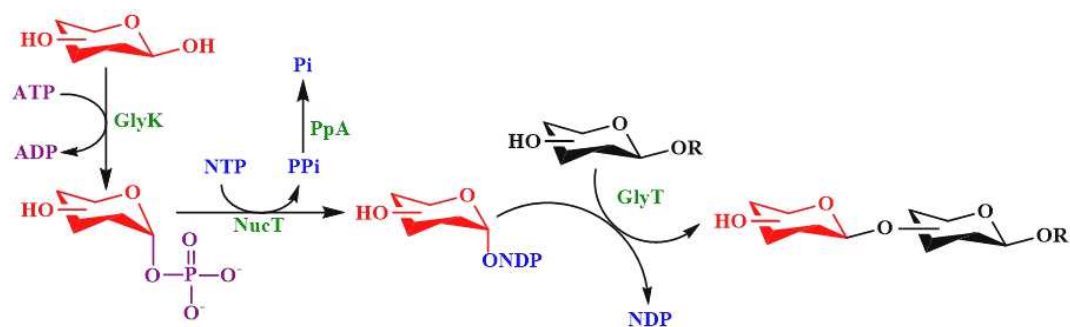
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630 **Fig. 3** The simplest routes for glycosyltransferase-catalyzed enzymatic synthesis of mammalian glycans with in
 631 situ generation of sugar nucleotides from a monosaccharide. Enzyme abbreviations: GlyK, glyco kinase (Yi et
 632 al., 2009); NucT, nucleotidyltransferase; GlyT, glycosyltransferase (Zhao et al., 2016); PpA, inorganic
 633 pyrophosphatase (Lau et al., 2010).

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Table 1 Natural functional oligosaccharides and their glycosidic linkages.

Type	Monosaccharides	Number of monosaccharides	Bonds indicative of functions
Arabino-oligosaccharides	Arabinose	2-8	α -1,5
Arabinogalacto-oligosaccharides	Arabinose, galactose	2-9	β -1,4
Arabinoxyl-oligosaccharides	Xylose, arabinose	5-10	α -1,2, α -1,3, β -1,4
Clycosylsucrose	Glucose, fructose	3	α -1,2, β -1,4
Cyclodextrins (CDs)	D-glucopyranose	6 (α -CD),7 (β -CD),8 (γ -CD)	α -1,4
Fructo-oligosaccharides	Sucrose, fructose	2-5	β -1,2
Galacto-oligosaccharides	Galactose	2-5	β -1,2, α -1,4
Galacturono-oligosaccharides	Galactosamine	2-9	α -1,4
Gentio-oligosaccharides	Glucose	2-10	β -1,6
Glucose-oligosaccharides	Glucose	2-10	α -1,2, β -1,3, β -1,6
Human milk oligosaccharides	Glucose, galactose, GlcNAc	2-8	α -1,2, α -1,3, α -1,4, α -2,3, β -2,6, β -1,3, β -1,4
Isomalto-oligosaccharides	Glucose	2-5	α -1,4
Lactosucrose	Galactose, fructose	2-3	β -1,4
Lactulose	Galactose, fructose	2	β -1,4
Malto-oligosaccharides	Mannitose, glucose	2-10	α -1,2, α -1,4
Palatinose	Glucose, fructose	2	β -1,6
Raffinose	Galactose, fructose, glucose	3	β -1,2, α -1,4
Rhamnogalacturon-oligosaccharides	Rhamnose, galactose	4-8	α -1,2, α -1,4, β -1,4
Soybean oligosaccharides	Fructose, galactose, glucose	2-4	α -1,6
Stachyose	Galactose, fructose, glucose	4	α -1,4
Xylo-oligosaccharides	Xylose	2-7	α -1,4

Table 2 Sources and applications of natural and glycan oligosaccharides based on the work of Patel & Goyal (2011).

Oligosaccharides	Natural occurrence	Applications	References
Isomalto-oligosaccharide	Starch from wheat, barley, potato, rice, cassava, honey, maltose, sucrose, dextran	Antidiabetic, prevent dental caries, stimulate the growth of colonic <i>Bifidobacterium</i> and <i>Lactobacilli</i>	Basu, Mutturi, & Prapulla, 2016; Bharti et al., 2015
Soybean oligosaccharides	Soybean seed	Competitive exclusion against potential pathogenic bacteria, reduction of oxidative stress, cardio-protective, chronic diseases prevention, amelioration insulin resistance	Fei, Ling, Hua, & Ren, 2014; Zhang, Cai, & Ma, 2015
Fructo-oligosaccharides	Antichoke, garlic, onion, asparagus, chicory, fermented beverage of plant extract, <i>Aspergillus</i> , <i>Fusarium</i> , <i>Arthrobacter</i> , <i>Aureobasidium</i> , <i>Gluconacetobacter</i> , <i>Bacillus</i> , <i>Saccharomyces</i>	Prebiotic activity, prevent urogenital infections, sweetener in beverages, acariogenic quality, effect on lipid metabolism, reduce risk of colon cancer, immunomodulatory property, antidiabetic activity	Kumar, Prashanth, & Venkatesh, 2014; Okada et al., 2010; Sanches Lopes et al., 2016; Wang et al., 2010; Wang, Li, & Wang, 2016
Lactulose	Cow milk	Used in treatment of hyperammonemia and portosystemic encephalopathy	Mussatto & Mancilha, 2007; Rentschler et al., 2015
Inulin	Chicory roots, onion, asparagus, antichoke	Function as dietary fiber, effect on lipid metabolism, reduction in risk of gastrointestinal diseases; absorption of calcium, magnesium and iron increased, stimulation of immune system	Apolinario et al., 2014; Shoaib et al., 2016; Yun, Choi, Song, & Song, 1999
Galacto-oligosaccharides	<i>Bifidobacterium bifidum</i> , <i>Kluyveromyces lactis</i> , <i>Sulfolobus solfataricus</i> ; Human milk, cow milk	Prebiotic	Goulas, Tzortzis, & Gibson, 2007; Kim, Park, & Oh, 2006
Gluco-oligosaccharides	<i>Leuconostoc mesenteroides</i> NRRL B-1299	Promote beneficial cutaneous flora	Iliev et al., 2008
Lactosucrose	<i>Pseudomonas aurantiaca</i>	Increase in <i>Bifidobacteria</i> population	Crittenden, & Playne, 1996; Kolida, & Gibson, 2007; Li et al., 2015; Silvério, Macedo, Teixeira, & Rodrigues, 2015
Malto-oligosaccharides	From starch by the action of pullulanase, isoamylase and amylases	Reduce the levels of <i>Clostridium perfringens</i> and family <i>Enterobacteriaceae</i>	Manas, Jonet, Murad, Mahadi, & Ilias, 2015
Xylooligosaccharides	<i>Aspergillus</i> , <i>Trichoderma</i> , <i>Penicillium</i> , <i>Bacillus</i> , <i>Streptomyces</i> , hardwood, corncob,	Prebiotic, antioxidant, gelling agent, treatment of diabetes, arteriosclerosis and colon cancer	Moure, Gullón, Domínguez, & Parajó, 2006; Samanta et al., 2015; Singh, Banerjee, & Arora,

	wheat straw, rice hull, barley straw		2015; Yang, 2016
Chitosan oligosaccharides	Depolymerised products of chitosan or chitin	Antioxidant, anti-tumor, anti-hypertensive, anti-microbial, fat-binding and hypocholesterolemic effects	Liu et al., 2010; Zou et al., 2015
Human milk oligosaccharides	Human milk	Facilitate preferential growth of <i>Bifidobacteria</i> and <i>Lactobacilli</i> , inhibition of lipopolysaccharide-mediated inflammation, enhancement of brain development	He et al., 2016; Wang, 2009
β -glucan oligosaccharide	Curdlan	Induction of monocytes to produce tumor necrosis factor alpha, stimulation of the secretion of interleukin 1b	Fu et al., 2015; Kumagai, Okuyama, & Kimura, 2016
Gentio-oligosaccharides	By digestion of starch; gentiobiose; <i>Penicillium multicolor</i>	Prebiotic	Côté, 2009; Fujimoto et al., 2009
Pectin-derived oligosaccharides	Higher plants; Sugar beet pulp	Prebiotic properties, amelioration diarrhoea, adsorption of calcium ions increased, antibacterial, antihyperlipidemic and antioxidant effects	Concha Olmosa & Zúñiga Hansen, 2012; Gómez, Gullón, Yáñez, Schols, & Alonso, 2016
Cyclodextrins	Transformation of starch by certain bacteria such as <i>Bacillus macerans</i>	Stabilization of deliquescent or volatile compounds in foods and chemicals, improvement poor aqueous solubility of drug compounds	Astray, 2009; Li et al., 2010; Radu, Parteni, & Ochiuz, 2016
Arabino-oligosaccharides	Sugar beet arabinan	Prebiotic	Westphal et al., 2010

Table 3 Distribution of oligosaccharides in human milk and bovine milk.

Abbreviation	Trivial name	Human milk (g L ⁻¹)*	Bovine milk (g L ⁻¹)*	Structure	Reference(s)
Neutral oligosaccharides					
2'-FL	2'-Fucosyllactose	0-3.8	-	Fuc α 1-2Gal β 1-4Glc	Baumgärtner et al., 2015;
3'-FL	3'-Fucosyllactose	0.04-1.1	-	Gal β 1-4(Fuc α 1-3)Glc	Boehm & Stahl, 2003;
DF-L	Difucosyllactose			Fuc α 1-2Gal β 1-4(Fuc α 1-3)Glc	Kulinich & Li, 2016; Kunz,
LNT	Lacto- <i>N</i> -tetraose	0.5-1.5	Trace	Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc	Rudloff, Baier, Klein, & Strobel, 2000;
LNnT	Lacto- <i>N</i> -neotetraose			Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc	Miyazaki, Sato, Furukawa, & Ajisaka, 2010;
LNFP I	Lacto- <i>N</i> -fucopentaose I	1.2-1.7	-	Fuc α 1-2Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc	Perret et al., 2005
LNFP-II	Lacto- <i>N</i> -fucopentaose II	0.3-1.0	-	Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc	
LNFP-III	Lacto- <i>N</i> -fucopentaose III	0.01-0.2	-	Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc	
LNFP-V	Lacto- <i>N</i> -fucopentaose V			Gal β 1-3GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc	
LNFP-VI	Lacto- <i>N</i> -fucopentaose VI			Gal β 1-4GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc	
LNDFH-I	Lacto- <i>N</i> -difucohexaose I	0.1-0.2	-	Fuc α 1-2Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc	
LNDFH-II	Lacto- <i>N</i> -difucohexaose II			Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc	
LNnDFH	Lacto- <i>N</i> -neodifucohexaose			Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc	
Para-LNnH	Para-Lacto- <i>N</i> -neohexaose			Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc	
LNnO	Lacto- <i>N</i> -neooctaose			Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc	
LNnFP V	Lacto- <i>N</i> -neofucopentaose V			Gal β 1-4GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)Glc	
LNH	Lacto- <i>N</i> -neohexaose			Gal(β 1,3)GlcNAc(β 1,3)[Gal(β 1,4)GlcNAc(β 1,6)]Gal(β 1,4)Glc	
Acidic oligosaccharides					
F-SL	3'Sialyl-3fucosyllactose			Neu5Ac α 2-3Gal β 1-4(Fuc α 1-3)Glc	Boehm & Stahl, 2003;
6'-SL	6'Sialyllactose	0.3-0.5	0.03-0.06	Neu5Ac α 2-6Gal β 1-4Glc	Jin, Joo, Li, Choi, & Han,
3'-SL	3'Sialyllactose	0.1-0.3		Neu5Ac α 2-3Gal β 1-4Glc	
LSTa	LS-Tetrasaccharide a	0.03-0.2	Trace	Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc	

LSTc	LS-Tetrasaccharide c	0.1-0.6	Trace	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc	2016; Neu et al., 2010; Tarr et al., 2015
Minor human milk oligosaccharides					
PI	BGA tetraose type 5			GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc	Kobata, 2010
PII	BGA hexaose type 1			GalNAc α 1-3(Fuc α 1-2)Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc	

645 BGA: Blood group A antigen; * The concentrations were compiled from previous studies (Bao, Chen, & Newburg, 2013; Gopal & Gill, 2000; Gwendolyn, Philip, Li, & Anita, 2013; Kunz & Rudloff, 2002; Sumiyoshi et al.,
646 2003).

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Table 4 The main properties and applications with different methods for oligosaccharide analysis.

Technique	Main Properties	Applications	References
HR-MS	Eelucidate molecular species without standard samples; Multiple stages of isolation and dissociation (MS ⁿ); Limited ionization capacity and low sensitivity for oligosaccharide analysis.	Chitosan oligosaccharides; Native and permethylated human milk oligosaccharides	Cederkvist et al., 2011; Oursel, Cholet, Junot, & Fenaille, 2017
ESI-MS-MS or MS ⁿ	Obtain information about the sequence, branching pattern and localization of modifications on oligosaccharides; Be valuable in the evaluation of isomeric oligosaccharides; Characterization of sulfated oligosaccharides ES-CID-MS/MS in the negative-ion mode.	Fructofuranosyl-containing gluco-oligosaccharides	Leijdekkers, Sanders, Schols, & Gruppen, 2011; Na et al, 2016; Tesić, Wicki, Poon, Withers, & Douglas, 2007; Veros, & Oldham, 2007; Zhang, Zhu, Zhang, Zhan, & Lin, 2014
NMR-ES-MS	Primary method for structural analysis of sulfated polysaccharide and derived oligosaccharides by NMR; Gain information about possible non-ionizable constituents; Large amounts of sample at milligram scale, quite time consuming, and a high level of expertise for NMR data interpretation.	Galactooligosaccharides	van Leeuwen, Kuipers, Dijkhuizen, & Kamerling, 2014
FT-ICR-MS	Ultra-high mass resolution and mass accuracy, non-destructive detection, high sensitivity and multistage MS ⁿ ; Identification at molecular-level analyses of organic mixtures without prior extraction or separation steps.	Thioxylo-oligosaccharide	Cederkvist et al., 2011; Jänis et al, 2005
MALDI-MS	Short analysis time, low fragmentation, wide mass range, salt and impurity tolerance of oligosaccharide analysis; Be difficult in sulfated oligosaccharides analysis due to the labile nature of the sulfate group.	Olive xylo-oligosaccharides	Reis, Coimbra, Domingues, Ferrer-Correia, & Domingues, 2002; Kim et al, 2016
MALDI-TOF-MS	Determination of the molecular masses of neutral and acidic oligosaccharides; Process of soft-ionization causes little or no fragmentation of analytes; A qualitative profile of the solubilized oligosaccharides; Not directly distinguish anomericity or branching configuration of oligosaccharides.	Arabinoxylo-oligosaccharides; Fructans; Chitosan oligosaccharides	Yang, Lee, Lee, Kim, & Kim, 2010; Sørensen, Pedersen, & Anastjuk, Shevchenko, Nazarenko, Dmitrenok, & Zvyagintseva, 2009; Chen, Zhu, Li, Guo, & Ling, 2010; Meyer, 2007; Park, Yang, Kim, & Kim, 2012; Suzuki et al., 2011
GPC-MALDI-TOF-MS	Determining the molecular weight of polymers by GPC; Less accurate molecular weight results for cationic polymers due to aggregation and ion exclusion.	High molecular weight oligomers	Liu, Maziarz, Heiler, & Grobe, 2003
GPC-ESI-ITMS	Detection of mono-disperse oligomers; Higher chromatographic resolution compared to GPC-MALDI-TOF-MS.	Low molecular weight oligomers	Liu, Maziarz, Heiler, & Grobe, 2003
HILIC-ELSD-MS ⁿ	Suitable for separation of highly polar carbohydrates; Detection of optical properties or functional groups of the analytes and compounds lacking chromophores.	Maltooligosaccharides; Labelled xyloglucans and xylan-derived oligosaccharides;	Leijdekkers, Sanders, Schols, & Gruppen, 2011
HILIC-TOF-MS	Faster separations with high fraction of organic solvent used in HILIC mobile phases, and higher desolvation within the MS source.	Xylo-oligosaccharides; Sake oligosaccharides	Ma, Sun, Chen, Zhang, & Zhu, 2014; Sastre, Ferreira, & Pedreschi, 2016; Tokuoka, Honda, Totsuka, Shindo, & Hosaka, 2017
HPAEC-PAD	Hydroxyl groups deprotonated to oxyanions under high pH for normal phase separation of oligosaccharides; Compatibility with gradient elution and picomolar sensitivity for oligosaccharide detection.	N-linked oligosaccharides	Arfelli, & Sartini, 2014; Cataldi, Campa, & De Benedetto, 2000; Maier et al., 2016
HPSEC-RI	Characterize the physicochemical properties of the interacting biopolymer fractions in detail; Be unapplied in gradient elution and sensitive to temperature by RI detector.	Xylo-oligosaccharide	

HPAEC-CE	Obtain the high resolution under physiological conditions; Minute quantities of samples with short analysis time; Require simple preparation and fluorescent derivatization of sample.	Sialylated oligosaccharides; Glycoprotein-derived oligosaccharides	Monti, Cattaneo, Orlandi, & Curadi, 2011
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658 HR-MS, high-resolution mass spectrometry; ES-MS-MS, electrospray tandem mass spectrometry; NMR, nuclear magnetic resonance spectroscopy; FT-ICR-MS, fourier transform-ion cyclotron resonance-mass spectrometry;
659 GPC, gel permeation chromatography; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; nESI-MS nano-electrospray ionization mass spectrometry; ITMS, ion trap mass
660 spectrometry; HPAEC, high performance anion exchange chromatography; PAD, pulsed amperometric detection; CE, capillary electrophoresis,
661 HILIC, hydrophilic interaction liquid chromatography; HPSEC, high performance size exclusion chromatograph.
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Table 5 Chemoenzymatic synthesis of oligosaccharides.

Product	Enzyme(s)	Yield (mg)	Yield (%)	Reference
<i>One-pot chemoenzymatic synthesis of α1-2-linked fucosides</i>				
Fuc α 1-2Gal β 1-3GlcNAc β ProN ₃	α 1-2Te2FT, Fkp, PmPpA	50.5	96	Zhao et al., 2016
Fuc α 1-2Gal β 1-3GlcNAc α ProN ₃	α 1-2Te2FT, Fkp, PmPpA	51.3	95	Zhao et al., 2016
Fuc α 1-2Gal β 1-3GalNAc α ProN ₃	α 1-2Te2FT, Fkp, PmPpA	35.7	95	Zhao et al., 2016
Fuc α 1-2Gal β 1-3GalNAc β ProN ₃	α 1-2Te2FT, Fkp, PmPpA	43.8	98	Zhao et al., 2016
LNFP I, Fuc α 1-2LNT	α 1-2Te2FT, Fkp, PmPpA	1146	95	Zhao et al., 2016
2'-Fucosyllactose, Fuc α 1-2Gal β 1-4Glc	GST- α 1-2-HpFucT	18	65	Albermann, Piepersberg, & Wehmeier, 2010
2'-Fucosyllactose-N ₃	GST-WbsJ	5.2	78	Li et al., 2008a,b
Fuc α 1-2Gal β -OMe	GST-WbsJ	4.4	71	Li et al., 2008a,b
T-antigen-OMe, β -D-Gal-(1-3)- α -D-GalNAc-OMe	GST-WbiQ	19	100	Pettit et al., 2010
Lewis ^y -tetrasaccharide	α 1-2-HpFucT, α 1-3-HpFucT ¹⁻⁴³³	4	45	Stein, Lin, & Lin, 2008
<i>One-pot chemoenzymatic synthesis of α1-3/4-linked fucosides</i>				
Lewis ^a -O-(CH ₂) ₈ CO ₂ CH ₃ or Lewis ^x -O-(CH ₂) ₈ CO ₂ CH ₃	α 1-3/4-HpFUCT ¹⁻⁴²⁸ , α 1-3-FucT ¹⁻⁴⁴¹	-	87-94	Ma et al., 2006; Ma, Simala-Grant, & Taylor, 2006
Lewis ^x	α 1-3-HpFucT Δ 52 FutA	-	95	Choi, Kim, Park, & Kim, 2016
3'-Fucosyllactose, Gal β 1-4-(Fuc α 1-3-)Glc	α 1-3-HpFucT Δ 52 FutA	-	96	Choi, Kim, Park, & Kim, 2016
Lewis ^x -ProN ₃	HhFT1, Fkp	25	63	Zhang et al., 2010
Sialyl Lewis ^x -ProN ₃	α 1-3-HpFucT ¹⁻⁴³³ , Fkp, iPPase	18.6	83	Soriano del Amo et al., 2010
LNFP III-ProN ₃	α 1-3-HpFucT Δ 52 FutA, FKP	109	92	Chen et al., 2015
LNDFH I, lacto-N-difuco-hexoase I	Commercial fucosyltransferase III (FUT3)	1.7	85	Miyazaki, Sato, Furukawa, Ajisaka, 2010
<i>One-pot chemoenzymatic synthesis of carbohydrates</i>				
LNT, Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc	<i>Aureobacterium</i> sp. L-101 lacto-N-biosidase	7.1	19-26	Murata, Inukai, Suzuki,

					Yamagishi, & Usui, 1999
LNT2, GlcNAc β 1-3Gal β 1-4Glc	NmLgtA, NmLgtB	1360	95%		Johnson, 1999
LNnT, Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc	NmLgtA, NmLgtB	1190	92		Johnson, 1999
LSTd, Neu5Ac α 2-3LNnT	<i>Trypanosoma cruzi</i> α 2-3-trans-sialidase	138	98		Yu et al, 2014
3'-SL, Neu5Ac α 2-3Gal β 1-4Glc	EcNanA, NmCSS, PmST1	68	68		Schmolzer, et al, 2015
DSLNnT	NmCSS, Pd2,6ST	236	99		Yu, et al, 2014
DSLac, Neu5Ac α 2-3(Neu5Ac α 2-6)Gal β 1-4Glc	NmCSS, Pd2,6ST	112	93		Yu, et al, 2014
DS'LNT, Neu5Ac α 2-6Gal β 1-3GlcNAc β 1-3(Neu5Ac α 2-6)Gal β 1-4Glc	NmCSS, Pd2,6ST	268	98		Yu, et al, 2014
Gb3 trisaccharide, Neu5Ac α 2-8Neu5Ac α 2-3Gal β 1-4Glc	NgLgtC	5000	75		Johnson, 1999
Gb4 tetrasaccharide	NgLgtD	1500	60		Johnson, 1999
<i>A whole-cell approach or engineered E. coli living-strategy</i>					
3'-SL, Neu5Ac α 2-3Gal β 1-4Glc	<i>Corynebacterium ammoniagenes</i> DN510 cells, <i>E. Coli</i> K12 CTP synthetase, <i>E. coli</i> K1 CMP-Neu5Ac synthetase, <i>N. gonorrhoeae</i> α 2-3-sialyltransferase	72,000	44		Endo, Koizumi, Tabata, & Ozaki, 2000
LNT-2, GlcNAc β 1-3Gal β 1-4Glc	<i>E. coli</i> JM109 (lacY+ lacZ-) with lgtA gene	6000	73		Priem, Gilbert, Wakarchuk, Heyraud, & Samain, 2002
LNnDFH, lacto-N-neodifucohexaose	NmLgtA, NmLgtB, <i>H. pylori</i> 26695 α 1-3-fucosyltransferase <i>FutA</i> and <i>RcsA</i>	1700	70		Dumon, Priem, Martin, Heyraud, Bosso, & Samain, 2001
LNFP II, lacto-N-neofucopentaose II	NmLgtA, NmLgtB, <i>H. pylori</i> 26695 α 1-3-fucosyltransferase <i>futB</i> gene	260	-		Dumon, Samain, & Priem, 2004
LNnFP V, Lacto-N-neofucopentaose V	NmLgtA, NmLgtB, <i>H. pylori</i> 26695 α 1-3-fucosyltransferase <i>futB</i>	280	-		Dumon, Samain, & Priem, 2004
Gal-(β 1-4)GlcNAc(β 1-3)Gal(β 1-4)[Fuc(α 1-3)]Glc					
Lewis ^X trisaccharide	<i>Helicobacter pylori</i> α 1-3-fucosyltransferase	2100	32		Koizumi, Endo, Tabata, Nagano, Ohnishi, & Ozaki, 2000
GM2, GalNAc β 1-4(NeuAc α 1-3)Gal β 1-4Glc	CMP-NeuAc synthase, α 2-3-sialyltransferase, UDP-GlcNAc C4	1250	-		Antoine, et al, 2003

	epimerase, β 1-4-GalNAc transferase			
GM1, Gal β 1-3GalNAc β 1-4(NeuAc α 1-3)Gal β 1-4Glc	β 1-3-galactosyltransferase	890	-	Antoine, et al, 2003
Gal β 1-4(Fuc α 1-3)GlcNAc β 1-4GlcNAc	<i>Rhizobium leguminosarum</i> chitin-synthase NodC and <i>Bacillus circulans</i> chitinase A1	620	-	Dumon, Bosso, Utille, Heyraud, & Samain, 2006
Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal	NmLgtA	1840	-	Dumon, Bosso, Utille, Heyraud, & Samain, 2006

682 EcNanA, *E. coli* sialic acid aldolase; FucT, fucosyltransferase; NmCSS, *Neisseria meningitidis* CMP-sialic acid synthetase; Pd2,6ST, *Photobacterium damsela* α 2-6-sialyltransferase; PmPpA, *Pasteurella multocida* inorganic
683 pyrophosphatase; PmST, *Pasteurella multocida* α 2-3-sialyltransferase; PmST1, *Pasteurella multocida* α 2-3-sialyltransferase 1; Psp2,6ST, *Photobacterium* sp. JT-ISH-224 α 2-6-sialyltransferase; NmLgtA, *Neisseria*
684 *meningitidis* β 1-3-N-acetylglucosaminyltransferase ; NmLgtB, *Neisseria meningitidis* β 1-4GalT; NgLgtC, *Neisseria gonorrhoeae* α 1-4-galactosyltransferase; NgLgtD, *Neisseria gonorrhoeae*
685 β 1-3-Nacetylgalactosaminyltransferase; Pd2,6ST, *Photobacterium damsela* α 2-6- sialyltransferase; HhFT1, *Helicobacter hepaticus* α 1-3-fucosyltransferase.

1. The biological functions of milk oligosaccharides, especially human milk oligosaccharides.
2. Developments in techniques for analysis of the oligosaccharide.
3. Advances in the oligosaccharides synthesis.

ACCEPTED MANUSCRIPT