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# Prebiotic Oligosaccharides: Special Focus on Fructooligosaccharides, Its Biosynthesis and Bioactivity

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**Abstract** The bacterial groups in the gut ecosystem play key role in the maintenance of host's metabolic and structural functionality. The gut microbiota enhances digestion processing, helps in digestion of complex substances, synthesizes beneficial bioactive compounds, enhances bioavailability of minerals, impedes growth of pathogenic microbes, and prevents various diseases. It is, therefore, desirable to have an adequate intake of prebiotic biomolecules, which promote favorable modulation of intestinal microflora. Prebiotics are non-digestible and chemically stable structures that significantly enhance growth and functionality of gut microflora. The non-digestible carbohydrate, mainly oligosaccharides, covers a major part of total available prebiotics as dietary additives. The review describes the types of prebiotic low molecular weight carbohydrates, i.e., oligosaccharides, their structure, biosynthesis, functionality, and applications, with a special focus given to fructooligosaccharides (FOSs). The review provides an update on enzymes executing hydrolytic and fructosyltransferase activities producing prebiotic FOS biomolecules, and future perspectives.

**Keywords** Prebiotics · Oligosaccharides · Fructooligosaccharide · Inulin · Kestose · Nystose · Levan

## Introduction

A microbial ecosystem in human gut plays crucial roles in digestion process [1]. The diverse microbial population in human digestive track, especially in colon, makes it metabolically

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more active. Colon, in the lower digestive tract, is involved in the breakdown of complex carbohydrates, dietary components, and some proteins that remains to be hydrolyzed in the upper digestive tract. The gut microbiota has a significant impact on human health; however, a major proportion, approximately 50%, of this microbial cosmos is non-cultivable [2–4]. It is important to modulate the gut microflora to enhance beneficial metabolic activities in the intestine. There are two possible ways to alter colonic microflora supplementation of live microbes, known as probiotics, or consumption of non-digestible food ingredients that stimulate growth and/or activity of microbes in the gut, called as prebiotics [5].

Prebiotics are non-digestible carbohydrates that are chemically stable at wide temperature and pH ranges [3]. Prebiotics are resistant to hydrolytic actions of intestinal enzymes but are fermentable by the intestinal beneficial microbes and, therefore, gives growth-promoting effects on beneficial microbes such as *Bifidobacterium* sp. and *Lactobacillus* sp. [2]. The colonic bacteria ferment these non-digestible dietary carbohydrates, producing a wide range of metabolites in gut, e.g., short-chain fatty acids [5]. The volatile short-chain (1–6 carbon), straight or branched, fatty acids exert crucial physiological implications, such as concomitant reduction of the luminal pH that inhibit the growth of pathogenic microorganisms, host signaling, and bacterial cross-feeding interactions [6–9].

Generally, all prebiotics fall under the category of dietary fibers but all dietary fibers are not prebiotic. The key prebiotics are inulin, fructooligosaccharides (FOSs), glucooligosaccharides (GuOSs), galactooligosaccharides (GaOSs), xylooligosaccharides (XOSs), maltooligosaccharides (MOs), isomaltooligosaccharides (IMOs), lactulose, lactosucrose, raffinose, stachyose, lactulosucrose, fructans, resistant starch, etc. [2, 6, 9] (Table 1). Inulin, FOS, and GaOS are the most popular prebiotics used in different food products, including baby foods [6]. The natural sources for prebiotics are plants, honey, and milk (Table 2). Prebiotics can be produced by enzymatic hydrolysis of polymers, e.g., oligofructose from inulin or by oligosaccharide synthesis through transglycosylation reactions [3, 4] (Tables 2 and 3). The structure of oligosaccharide, including the nature of gylcosidic bonds, its solubility, viscosity, fermentability, and degree of

**Table 1** Types of oligosaccharides and their composition

| | Category | Molecular sequence | Nature of glycosidic bonding |
|---|---|---|---|
| 1. | Fructooligosacharides | $G\text{-}(F)_n$ | β (2–1)/β (2–6) |
| 2. | Galactooligosacharides | $G\text{-}(Ga)_n$ | β (1–3) and β (1–4) |
| 3. | Gentiooligosacharides | $(G)_n$ | β (1–6) |
| 4. | Isomaltooligosacharides | $(G)_n$ | α (1–6) |
| 5. | Isomaltulose | $(G\text{-}F)_n$ | α (1–6) |
| 6. | Lactosucrose | Ga-G-F | β (1–4) and α (1–2) |
| 7. | Lactulose | Ga-F | β (1–4) |
| 8. | Maltooligosacharides | $(G)_n$ | α (1–4) |
| 9. | Raffinose | Ga-G-F | α (1–6) and α (1–2) |
| 10. | Cyclodextrin | $(G)_n$ | Cyclic α (1–4) |
| 11. | Xylooligosacharides | $(X)_n$ | β (1–4) |
| 12. | Stachyose | $(Ga)_2\text{-}G\text{-}F$ | α (1–6) and α (1–2) |
| 13. | Arabino-oligosaccharides | (A)n | α-(1,5) |
| 14. | Chitosanoligosacharides | $(GlcNAc)_n$ | β (1–4) |
| 15. | Pectin derived oligosacharides | $(GalA)_n$ | α (1–4) |
| 16. | Agarooligosaccharide | $(Gal)_n$ | β (1–4) and α (1–3) |
| 17. | Maltosylsucrose | $F(G)_2$ | $^6$F α (1–6)/$^6$G α (1–6) |

*A* arabinose, *G* glucose, *F* fructose, *Ga* galactose, *X* xylose, *GlcNAc N*-acetylglucosamine, *GalA* galacturonic acid

**Table 2** Natural sources for oligosaccharides

| Oligosaccharides | Natural resources | References |
|---|---|---|
| Frcutooligosacharides | Asparagus, sugar beet, garlic, chicory, onion, Jerusalem artichoke, wheat, honey, banana, barley, tomato, and rye are special sources of fructooligosaccharides | [10, 11] |
| Galactosylsucroses | Soyabean and other pulses | [12] |
| Raffinose-type oilgosacharides (raffinose, stachyose, and verbascose) | Soyabean and other pulses | [13] |
| Xylooligosaccharides | Bamboo shoots, husks, straws, and corncobs | [12, 14] |
| Galactooligosaccharides | Milk | [15] |
| Isomaltulose | Honey, sugarcane juice, and food-grade molasses | [16] |
| Pectin | Citrus peel, apple pomace, or sugar beet, while inulin and fructans are extracted from chicory or agave | [17] |

polymerization (DP) are important coordinates for determining the functionality of a prebiotic [4]. Prebiotic oligosaccharides are helpful in minimizing health-related risks, such as diabetes, cardiovascular disorders, cancer, acute infection, inflammation, and obesity. Prebiotic consumption also enhances bioavailability of nutritionally important minerals such as calcium, magnesium, and iron [33]. Different probiotic microbes employ different genetic mechanisms for utilizing different types of oligosaccharides; therefore, the extent of efficacy of dietary fibers on health is dependent on the type of prebiotic biomolecule consumed [34].

The aim of the present review is to summarize the background information pertaining to different prebiotic oligosaccharides with a major focus on fructooligosaccharides.

## Types of Prebiotic Oligosaccharides

### Galactooligosaccharides

Galactooligosaccharides are galactose-containing oligosaccharides having β (1–3) and β (1–4) bonds among the monomers, synthesized by transgalactosylase activity of β-galactosidase enzyme utilizing lactose [35]. It has a bifidogenic property with a profound effect on

**Table 3** Microorganisms reported to secrete Inulinases

| Type of inulinase | Organism | References |
|---|---|---|
| Exoinulinase | *Bacillus polymyxa* MGL21 | [18] |
| | *Bacillus licheniformis* | [19] |
| | *Bifidobacterium longum* | [20] |
| | *Bacillus* sp. Snu7 | [21] |
| | *Geobacillus stearothermophilus* | [22] |
| | *Kluyveromyces marxianus* | [23] |
| | *Penicillium* sp. TN-88 | [24] |
| | *Pseudomonas mucidolens* | [25] |
| | *Thermotoga maritima* | [26] |
| Endoinulinase | *Aspergillus awamori* | [27] |
| | *Aspergillus ficuum* | [28] |
| | *Arthrobacter* sp. S37 | [29] |
| | *Aspergillus niger* | [30] |
| | *Penicillium* sp. TN-88 | [31] |
| | *Penicillium purpurogenum* | [32] |

*Bifidobacterium* sp. level in the gut [36]. It can tolerate high temperature and low pH and, therefore, is preferred additive in food products [35]. It is also used in infants' milk as functional ingredients exerting health-promoting effects. The mixture of 90% short-chain galactooligosaccharides along with 10% long-chain fructooligosaccharides are used in human milk to mimic the molecular size distribution of natural oligosaccharides [37, 38].

## Xylooligosaccharides

Xylooligosaccharide is the new emerging prebiotics used as functional food products in pharmaceutical, nutraceutical, and agriculture sectors [39]. Xylooligosaccharide is made up of xylose monomeric units having β (1–4) glycosidic linkage. The xylan and arabinoxylan polysaccharides, widely distributed in plants, are key feedstock for xylooligosaccharide production. Xylooligosaccharide can be synthesized by enzymatic hydrolysis or chemical fractionation of lignocellulosic materials [40]. At present, xylooligosaccharide is commercially produced from corn cob employing xylanase enzyme [4].

## Malto- and Isomaltooligosaccharides

Maltooligosaccharides are composed of a chain of glucose monomers linked with α (1–4) linkages. These can be produced from polysaccharides such as starch, glycogen, and amylose by the catalytic actions of debranching enzymes, amylases, and pullulanase. Maltooligosaccharides are susceptible to the intestinal enzymes and are generally absorbed in the small intestine. Though maltooligosaccharides are less effective in the growth of *Bifidobacteria*, they can reduce the level of putrefactive bacteria in the intestine [41]. Isomaltooligosaccharides (IMO) are oligomers of glucose with generally α (1–6) bonds, and sometimes with α (1–2) or α (1–3) or α (1–4) linkages. The IMO include different oligomers, e.g., isomaltose, panose, isomaltotriose, isomaltotetraose, isomaltopentaose, nigerose, kojibiose, and other highly branched oligosaccharides. Traditionally, IMO is synthesized from starch by enzymatic actions of α-amylase, β-amylase, or pullulanase, generating maltose and maltotriose, followed by transglycosylation activity by α-transglucosidase [42]. Basu et al. established simultaneous saccharification and transglucosylation approach for production of isomaltooligosaccharides from starch [43]. However, this approach generally yields a mixture of glucooligosaccharides containing both α (1–6) and α (1–4) glycosidic bonds [44].

Transglycosidation reaction by glucosyltransferases also results biosynthesis of IMO. Glucose moiety is transferred to its accepter molecules such as maltose or isomaltose or O-α-methylglucoside to produce glucooligosaccharide. *Leuconostoc mesenteroides* is a well-known microbe to produce enzymes, such as mutansucrases, dextransucrases, alternansucrases, or reuteransucrases, with catalytic efficiency of glucooligosaccharide biosynthesis [45–47].

Dextransucrase catalyzes hydrolysis of sucrose, followed by transfer of glucose unit from sucrose to acceptor molecules, synthesizing IMO [48]. In the presence of suitable acceptor molecules, such as maltose or isomaltose, dextransucrase catalyzes IMO synthesis. This approach yields oligosaccharides with nearly exclusive α (1–6) glycosidic bonds among glucose moieties [49]. In addition, dextransucrase also catalyzes polysaccharide (dextran) synthesis using the growing glucan chain as acceptor [45]. A continuous synthesis process has been developed for production of IMO and oligodextrans from sucrose using dextransucrase and dextranase enzymes [50].

### Gentiooligosaccharide

Gentiooligosaccharide is composed of glucose units linked with β (1–6) bond. Apart from being prebiotic, its bitter taste is useful to generate a specific taste in certain beverages. Gentiooligosaccharide biosynthesis is catalyzed by two methods, transglycosylation and glycosyltransferase activities. The combination of β-glucosidase, which produces gentiotriose from gentiobiose, and β (1–6) glucanase enzymes, in the case of which gentiotriose is both donor and acceptor, can efficiently synthesize gentiooligosaccharides [51, 52].

Gentiooligosaccharides may also be synthesized by glycosyltransferase reactions executed by dextransucrase taking sucrose as substrate, in presence of gentiobiose (acceptor) molecules [53]. Further, gentiooligosaccharides can be obtained by hydrolysis of lichen polysaccharide, pustulan [54]. Gentiooligosaccharides enhances the growth of *Bifidobacterium infantis* and *Lactobacillus acidophilus* in the gut and have almost similar digestibility in comparison to the standard prebiotics.

### Chitosan Oligosaccharides and Cyclodextrins

Chitosan is a derivative of chitin, composed of D-glucosamine and *N*-acetyl-D-glucosamine units joined by β (1–4) glycosidic linkages. Chitosan oligosaccharides are produced by chemical (high temperature and low pH) or enzymatic (chitosanases) hydrolysis of the chitosan polysaccharides [55]. Their consumption promotes the growth of colon bacteria (e.g., *Bifidobacterium* sp.), therefore, useful in the food industry [56]. Additionally, chitosan oligosaccharides and cyclodextrins exert various biomedical benefits such as antitumor, antimicrobial, anti-inflammatory, antioxidant, and immuno-enhancing effects [57].

Cyclodextrin is a cyclic oligosaccharide composed of glucose molecules linked with cyclic α (1–4) bonds. It is biosynthesized from starch by the amylolytic enzyme, cyclodextrin glucosyltransferase, which catalyzes transglycosylation, intramolecular cyclization, and intermolecular coupling reactions [51].

### Pectin-Derived Oligosaccharides

The complex heteropolysaccharide, pectin, has been found to have the potential of a prebiotic substance. The oligosaccharides are produced by partial depolymerization of pectin by acid or enzymatic hydrolysis, hydrothermal processing, and physical degradation [58, 59]. Pectic oligosaccharide is composed of D-galacturonic acid (GalA) units, present in either its acetylated or methylated forms, linked by α (1–4) bonds. They include several categories of oligosaccharides such as galactooligosaccharide, arabinogalactooligosaccharide, rhamnogalacturonooligosaccharide, oligogalacturonide, and arabinooligosaccharide. Apart from having prebiotic potential, these oligosaccharides exhibit antiulcer, anticancer, antiobesity, and anti-inflammatory properties [60].

The pectin from sugar beet is known to be rich in arabinan fraction [61]. Endoarabinanases are employed to hydrolyze arabinan into arabinooligosaccharides. The L-arabinosyl residues are linked with α (1–5) or α (1–2) or α (1–3) bonds [62]. It has been observed to significantly increase the population of beneficial gut microbes such as *Bifidobacterium* sp. [63]. Endogalactanases can generate arabinogalactooligosaccharide from soybeans, rhamnogalacturonase can produce rhamnogalacturonooligosaccharide from apple, and arabinoxylooligosaccharide can be made by employing xylanases on wheat biomass [55, 64].

### Agarooligosaccharide and Neo-agarooligosaccharide

Agarooligosaccharide and neo-agarooligosaccharide are produced from agar by hydrolytic activities of α-agarase and β-agarase enzymes [65]. In agarooligosacharides, 3,6-anhydro-L-galactose and D-galactose units are linked by α (1–3) and β (1–4) glycosidic bonds. Agarooligosaccharide and neo-agarooligosaccharide are able to boost the growth of gut bacteria such as *Bifidobacteria* sp. and *Lactobacilli* sp. [66]. They also exhibit other health-promoting activities, such as anti-inflammatory, antitumor, and antioxidant properties [65].

### Raffinose, Stachyose, and Verbascose

Raffinose, stachyose, and verbascose are tri-, tetra-, and pentasaccharides, respectively. These oligosaccharides are found in *Glycine max* in substantial amount (e.g., 2–6% raffinose and 1–2% stachyose (*w*/*w*) of dry mass). In these oligosaccharides, galactose units are linked to sucrose by α (1–6) glycosidic bonds. Raffinose is composed of galactose, glucose, and fructose, whereas stachyose contains two galactose, one glucose, and one fructose molecule. The raffinose and stachyose are biosynthesized from sucrose by galactosyltransferases enzymes known as raffinose synthase and stachyose synthase, respectively [67]. Verbascose is composed of three galactose, and one glucose and fructose molecules. Stachyose synthase catalyzes synthesis of verbascose. The soybean oligosaccharides are generally obtained from soybean whey, a by-product generated during production of soy protein [40]. The soybean oligosaccharides are considered as effective prebiotic biomolecules for functional food applications.

### Lactulose, Lactosucrose, Glycosylsucrose, and Isomaltulose

Lactulose is a ketose disaccharides synthesized from lactose, in which glucose moiety is isomerized into fructose. In lactulose, the units are joined by β (1–4) glycosidic linkage. It can be synthesized by catalytic actions of β-galactosidase and glucose isomerase enzymes using whey lactose [68]. In milk, lactose can also be transformed into lactulose by moderate heating [69]. It is not digestible in the intestine and can stimulate growth of *Lactobacilli* sp. and *Bifidobacteria* sp. bacteria; on the other hand, it decreases the growth of clostridia, coliforms, streptococci, and bacteroides in the gut [4].

Lactosucrose (4(G)-beta-D-galactosylsucrose) is composed of galactose, glucose, and fructose with β (1–4) and α (1–2) glycosidic bonds. The trisaccharide is synthesized by transfructosylation reaction catalyzed by levansucrase or β-fructofuranosidase enzyme utilizing lactose and sucrose [70]. Lactosucrose enhances mineral absorption in the gut and also aid in growth of gut microbiota. It inhibits intestinal lipid absorption and, thus, helps in preventing obesity. It is preferred to be used as an ingredient in desserts, sweets, confectioneries, yogurts, tea, coffee, etc. [71].

Glycosylsucrose, also known as coupling sugar, is a trisaccharide produced from sucrose and maltose via the transglycosylation activity of the cyclomaltodextrin glucanotransferase enzyme. Unlike sucrose, it does not cause dental caries and extends other benefits to food processing applications. However, it is lesser bifidogenic responsive as compared to other prebiotics [40, 41].

Isomaltulose, also known as palatinose, is a disaccharide in which glucose and fructose are linked by α (1–6) glycosidic linkages. Thus, it is a structural isomer of sucrose, synthesized via rearrangement of the glycosidic linkage in sucrose. It naturally occurs in honey and sugarcane juice and is considered to be a potential prebiotic molecule [40, 51].

### Fructooligosaccharides

Fructooligosaccharide is one of the most explored prebiotics [72]. The low-calorie FOS, besides being prebiotic, helps in reducing cholesterol level, inhibiting the growth of harmful bacteria and improving mineral absorption in the gut. FOS is composed of one glucose moiety followed by fructose moieties ranging from 2 to 60 linked by β (2–1) or β (2–6) glycosidic bonds, for example, 1-kestose (one glucose and two fructose), nystose (one glucose and three fructose), and fructofuranosyl nystose (one glucose and four fructose) [73, 74]. FOS is not digested in the small intestine but gets metabolized in cecum into small-chain fatty acid and L-lactate, and other bioactive molecules beneficial to human health [2]. FOS enhances absorption of minerals such as $Mg^{+2}$ and $Ca^{+2}$ and decreases fatty acid level in the gut [75]. As fructan biosynthesis occurs in plants and fungi, besides in bacteria, the potential plant sources of FOS are *Triticum* sp., *Allium cepa*, *Allium sativum*, *Secale cereal*, *Solanum lycopersicum*, *Asparagus officinalis*, *Cichorium intybus*, *Musa* sp., and *Helianthus tuberosus* [4, 76]. The commercial market of prebiotics is at present dominated by inulin (polymer of fructose), FOS, GaOS, and IMO. The inulin, oligofructose (produced by hydrolysis of inulin), or FOS are extensively studied prebiotics, and several reports suggest that use of fructans in diet stimulates gut microbes more effectively. FOS is commercially used as a food ingredient and publically available under several trade names like Neosugar, NutraFlora®, Meioligo®, and Actilight® [72].

## Fructooligosacharides: Properties, Synthesis, and Applications

### Physiochemical Properties of Fructooligosaccharide

FOS, being water soluble, hygroscopic and relatively less sweet, and of reduced caloric value and prebiotic in nature, are useful ingredients in food industry [77]. Sweetness of FOS decreases with increase in the degree of polymerization [78]. FOS is composed of fructose monomers linked together with a terminally attached glucose unit, i.e., glucose-$(\text{fructose})_n$ (Fig. 1). On the basis of chemical structure, FOS may be categorized in different types, such as inulin-FOS ($^1$F-FOS), levan FOS ($^6$F-FOS), and neo-FOS ($^6$G-FOS) [64, 79]. These types differ by the nature of glycosyl bonds among the monomer units, for example, fructose units are linked by linear β (2–1) and β (2–6) in inulin- and levan-type FOS, respectively. Commercially available inulin-FOS includes 1-kestose ($^1$F-β-D-fructofuranosylsucrose) (GF2), 1-nystose [$^1$F ($^1$F-β-D-fructofuranosyl)$_2$ sucrose] (GF3), and $^1$F-β-fructofrcutofuranosyl nystose (GF4). In neo-FOS, glucose moiety of sucrose is linked to fructose unit via β (2–6) linkage in branched manner, which generates the possibilities for elongation of fructose chain in both β (2–1) and β (2–6) orientations [79]. Examples of neo-FOS are neo-kestose (neo-GF2), neo-nystose (neo-GF3), and neo-frcutofuranosyl nystose (neo-GF4). Neo-FOS has superior prebiotic activity as well as stability in comparison to $^1$F-FOS [80].

For decades, researchers are engaged in mining the strategies for production of FOS [81]. Fructans have been successfully extracted from plant sources such as *Cichorium intybus* (chikori), *Allium cepa* (onion), and *Helianthus tuberosus* (Jerusalem artichoke) for FOS production. However, isolation and downstream processing of FOS from these natural sources is tedious and costly (Table 4) [10]. The stereospecificity of glycosidic bond formation between the monomeric units of FOS makes its chemical synthesis a challenging process [95]. Biotechnological approaches can provide an efficient and productive platform for FOS

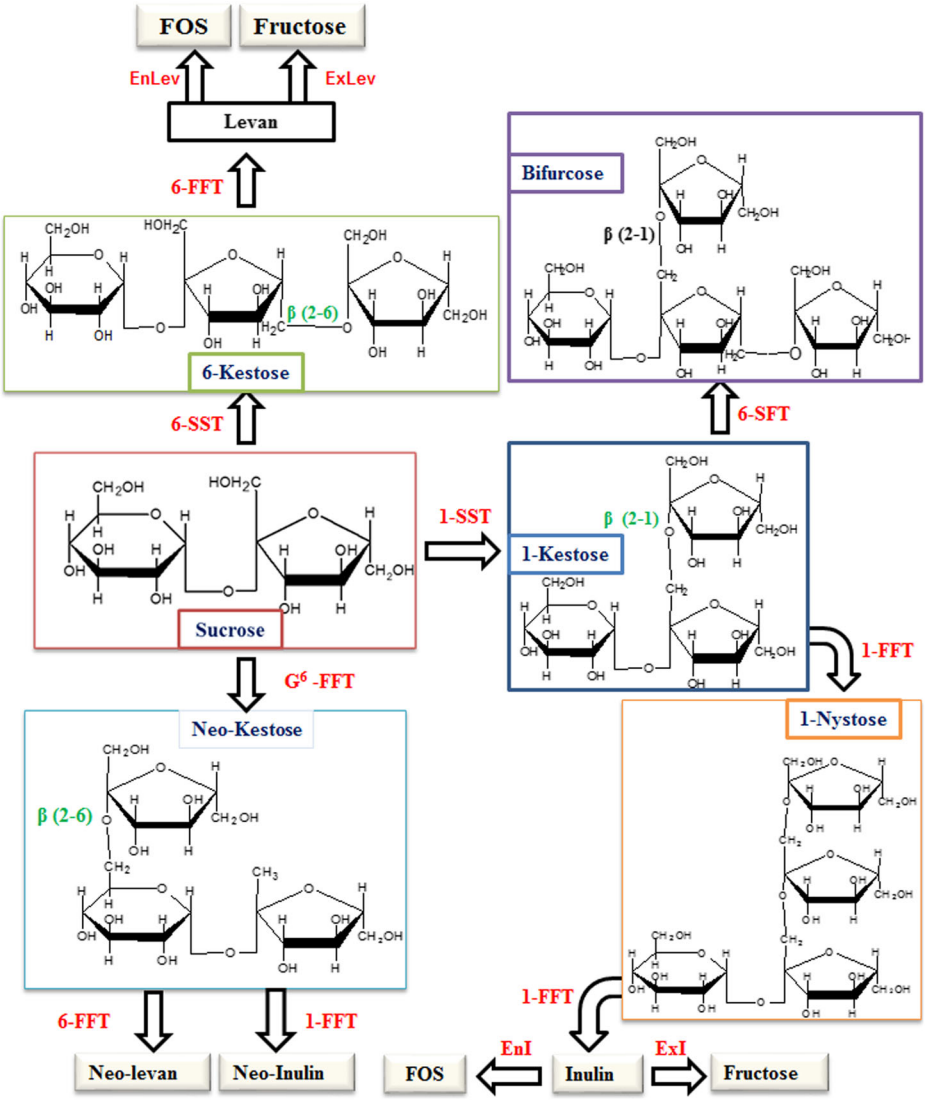


**Fig. 1** An overview of reactions catalyzed by FTases for synthesis of fructooligosaccharides and biopolymers (levan and inulin). Enzymes are highlighted in red color. 1-SST sucrose:sucrose 1-fructosyl-transferase, 1-FFT fructan:fructan 1-fructosyltransferase, 6-SST sucrose:sucrose 6-fructosyltransferase, 6-FFT fructan:fructan 6-fructosyltransferase, $G^{6-}$FFT fructan:fructan 6G-fructosyltransferase, 6-SFT sucrose:fructan 6-fructosyltransferase, ExI exoinulinases, EnI endoinulinase, EnLev endolevanase, ExLev exolevanase

production. Here, we have reviewed the sources having genetic potential for biosynthesis of FOS at pilot scale.

## Biotechnological Approach for Efficient Production of Oligosaccharide

The enzymatic method for production of FOS is a cost- and time-effective process that can be exploited at industrial scale. The enzymatic sources have been reported from different

**Table 4** List of plants having fructosyltransferase enzyme

| | | |
|---|---|---|
| 1. | *Agave americana* (agave) | [82] |
| 2. | *Agave tequilana* Weber Blue variety | [83] |
| 3. | *Agave Vera cruze* (agave) | [84] |
| 4. | *Allium cepa* (onion bulbs) | [85] |
| 5. | *Arabidopsis thaliana* | [86] |
| 6. | *Asparagus officinalis* (asparagus root) | [87] |
| 7. | *Hordeum vulgare* (barley) | [88] |
| 8. | *Cichorium intybus* (chicory) | [89] |
| 9. | *Helianthus tuberosos* (Jerusalem artichoke) | [90] |
| 10. | *Lactuca sativa* L. (lettuce) | [91] |
| 11. | *Lolium perenne* L. | [92] |
| 12. | *Lycoris radiata* | [93] |
| 13. | *Phleum pratense* | [94] |

organisms catalyzing the biosynthesis of FOS with different degree of polymerization and/or glycosidic linkages (Tables 4, 5, and 6). The following methods of catalytic production of FOS have been explored.

*Biosynthesis of Fructooligosaccharide from Sucrose*

The enzymes that execute catalytic biosynthesis of FOS using sucrose as substrate are known as frutosyltransferases (EC 2.4.1.99) or β-fructofuranosidases (EC 3.2.1.26) [138]. Even after a long debate, still the dilemma exists in the nomenclature of these enzymes. Fructosyltransferases (FTases) catalyze the transfructosylation of sucrose, which includes hydrolysis of sucrose molecule followed by transfer of liberated fructose unit to an acceptor molecule such as sucrose or other fructooligosaccharide like molecule. FTases break α (1–2) linkage in sucrose and transfer the liberated fructose molecule at either β (2–1) or β (2–6) position of fructose unit of another sucrose molecule. Generally, the transfer of fructose moiety does not happen to the free glucose or water molecules present in the reaction mixture. At the end, the reaction mix contains several components including some unreacted sucrose, glucose,

**Table 5** List of fungi having fructosyltransferase activity

| | Organism | Source of enzyme | References |
|---|---|---|---|
| 1. | *Aureobasidium* sp. P6 | Intracellular | [96] |
| 2. | *Aspergillus aculeatus* | Extracellular | [97] |
| 3. | *Aspergillus flavus* | Extracellular | [98] |
| 4. | *Aspergillus foetidus* | Extracellular | [99] |
| 5. | *Aspergillus japonicus* | Intra- and/or extracellular | [100] |
| 6. | *Aspergillus niger* | Extracellular | [101] |
| 7. | *Aspergillus oryzae* | Extracellular | [102] |
| 8. | *Aspergillus phoenicis* | Intracellular | [103] |
| 9. | *Aspergillus sydowi* | Intracellular | [104] |
| 10. | *Aspergillus terreus* | Extracellular | [98] |
| 11. | *Aureobasidium pullulans* | Intra- and extracellular | [105] |
| 12. | *Penicillium citrinum* | Intra- and/or extracellular enzyme | [106] |
| 13. | *Penicillium islandicum* | Extracellular | [98] |
| 14. | *Penicillium purpurogenum* | Intra- and extracellular enzyme | [107] |
| 15. | *Penicillium rugulosum* | Extracellular | [108] |
| 16. | *Rhodotorula* sp. | Extracellular | [109] |
| 17. | *Xanthophyllomyces dendrorhous* | Extracellular | [110] |

**Table 6** List of bacteria having fructosyltransferase activity

| | Organism | Source of enzyme | References |
|---|---|---|---|
| 1. | *Actinomyces naeslundii* | Intracelluar | [111] |
| 2. | *Actinomyces viscosus* | Extracellular | [112] |
| 3. | *Arthrobacter* K-1 | Extracellular/intracellular | [113] |
| 4. | *Bacillus amyloliquefaciens* | Intra- and extracellular FTase | [114] |
| 5. | *Bacillus macerans* | Extracellular | [115] |
| 6. | *Bacillus megaterium* | Intracellular | [116] |
| 7. | *Bacillus methylotrophicus* SK 21.002 | Extracellular | [117] |
| 8. | *Bacillus subtilis* | Intracelluar | [118] |
| 9. | *Bacillus subtilis BB04,* | Extracellular | [119] |
| 10. | *Bacillus subtilis* NRC 33a | Extracellular | [120] |
| 11. | *Brenneria goodwinii* | Intracelluar | [121] |
| 12. | *Clostridium acetobutylicum* | Intracellular | [122] |
| 13. | *Clostridium arbusti* SL206 | Intracelluar | [123] |
| 14. | *Erwinia amylovora* | Extracellular | [124] |
| 15. | *Gluconacetobacter diazotrophicus* | Intra- and extracellular FTase | [81] |
| 16. | *Lactobacillus sanfranciscensis TMW 1.392* | Intracelluar | [125] |
| 17. | *L. sanfranciscensis LTH 2590* | Extracellular | [126] |
| 18. | *Lactobacillus panis* | Intracellular | [127] |
| 19. | *Lactobacillus reuteri* | Intra- and/or extracellular FTase | [128] |
| 20. | *Lactobacillus sanfranciscensis* | Intracelluar | [125] |
| 21. | *Leuconostoc citreum Strain BD1707* | Extracellular | [129] |
| 22. | *Leuconostoc mesenteroides* | Intracelluar | [130] |
| 23. | *Paenibacillus bovis sp.* nov BD3526 | Extracellular | [131] |
| 24. | *Pediococcus acidilactici* | Extracellular | [132] |
| 25. | *Pseudomonas syringae pv. phaseolicola* | Extracellular | [133] |
| 26. | *Rahnella aquatilis* | Intracelluar | [134] |
| 27. | *Streptococcus mutans* | Extracellular | [135] |
| 28. | *Streptococcus salivarius* | Extracellular/intracellular | [136] |
| 29. | *Zymomonas mobilis* | Extracellular | [137] |

fructose, and oligosaccharides of different degree of polymerization. The nature of FOS may also vary under variable reaction conditions. Some FTases catalyze synthesis of FOS only, but not the polymer [79, 81, 138]. FTase from *Aspergillus aculeatus* catalyzes the synthesis of short-chain FOS (kestose, nystose, and fructosylnystose), while levansucrase not only synthesizes FOS but also produce polymers of fructose, called as levan [97, 121]. FTases possess both hydrolytic and transfructosylation activities, depending upon the concentration of sucrose. In case of high sucrose concentration, increased transfructosylation activity has been observed [139]. The extent of hydrolyitc or transfructosylating activities differs with different enzymes. FTases have been reported in plants, bacteria, and fungi.

**Plant Fructosyltransferase** About 15% flowering plants have the mechanism for biosynthesis of fructans, the linear or branched chain of fructose (Table 2) [140]. Depending on the nature of firstly synthesized triscaharide, these are divided into five distinct groups: (1) inulin (Astarales), (2) levan (Poales), (3) neo-inulin (Asparagales), (4) neo-levan (Poales), and (5) graminans (Poales) [141]. For synthesis of inulin-type fructans in plants, two enzymes are required: (i) sucrose, sucrose 1-fructosyl-transferase (1-SST); (ii) fructan, fructan 1-fructosyltransferase (1-FFT) [90]. 1-SST hydrolyzes one sucrose molecule and transfers the fructose moiety onto fructose of another sucrose molecule, consequently forming a trisaccharide, 1-kestose (DP-3). Thus, two molecules of sucrose are utilized in this mechanism.

Another enzyme, 1-FFT, transfers fructose to other fructan molecules (e.g., 1-kestose and inulin) at the fructose moiety of the 1-kestose, generating 1-nystose (DP-4), or oligosaccharide of higher DP, and subsequently inulin (DP ≥ 25). Thus, 1-FFT functions through breaking and reconstructing the β (2–1) linkage (Fig. 1) [142]. The preferred donor substrate for 1-FFT could be 1-kestose and inulin. Genes encoding 1-SST and 1-FFT enzymes have been identified in plants [83, 143]. In plants, which synthesizes levan-type fructans, enzymes are yet to be fully explored. Plants possess either single enzyme, sucrose:fructan 6-fructosyltransferase (6-SFT), or sometimes more than one enzymes that may be more specific in their functions. For example, sucrose:sucrose 6-fructosyltransferase (6-SST) catalyzes the synthesis of 6-kestose, and fructan:fructan 6-fructosyl-transferase (6-FFT) is responsible for the synthesis of levan-type fructan through elongation of 6-kestose by transferring fructose moieties [144]. Existence of 6-SFT in plants is reported, but there is uncertainty about the 6-SST/6-FFT [144, 145]. Another enzyme is fructan:fructan 6-glucose-fructosyl-transferase ($G^6$-FFT), which produces neo-type fructans by transferring glucose unit to fructose moiety through β (2–6) linkage [73, 146]. In plants, fructan synthesis occurs via a complex process. Further, it is difficult to isolate and purify the enzymes of plant origin, although attempts have been made for isolation and purification of enzymes from plants such as from asparagus, onion, and artichoke [143, 147]. Heterologous expression of plant genes in microbial cells could be one way for industrial application of these genes; however, heterologous expression of plant genes faces challenges related to codon optimization and appropriate protein folding [148, 149].

**Fungal and Bacterial Fructosyltransferases** Since last three decades, many fungal and bacterial species have been explored that express FTases [96, 106]. Interestingly, microbial FTases have both hydrolytic and transferase activities within one enzyme [11]. Several fungal organisms are reported to have FTase activity, such as *Aureobasidium pullulans* (CFR 77), *Aureobasidium* sp. P6, *Aspergillus japonicas*, *Schwanniomyces occidentalis*, *Penicillium citrinum*, and *Xanthophyllomyces dendrorhous* [96, 100, 106, 110, 150, 151]. In Tables 5 and 6, fungal and bacterial sources for FTase have been presented. However, specificity and properties of the enzyme vary from one species to another species (Tables 5 and 6). Generally, FTases from bacterial sources have ~ 45–65-kDa molecular weight, but in lactic acid-producing bacteria (LAB), FTases are of high molecular weight ranging from 60 to 170 kDa [152, 153]. Many FTases are isolated and characterized from the probiotic lactic acid-producing bacteria such as *L. reuteri*, *L. johnsonii*, and *L. gasseri.* Recently, the newly identified group of levansucrase enzyme in fungi and bacteria (EC 2.4.1.10) attracted attention for the catalytic synthesis of β (2–6) linked FOS and levan with higher DP using sucrose as substrate [154]. Levansucrases have been isolated and characterized from different gram-positive and gram-negative bacteria. Many LAB have been found to have FTases, such as *Streptococcus salivarius*, *Leuconostoc mesenteroids*, *Leuconostoc citreum Strain BD1707*, and *Lactobacillus reuteri* [129, 152]. Some non-lactic acid-producing bacteria (NLAB) representing levensucrase production are *Bacillus polymyxa*, *Zymomonas mobilis*, *Erwinia amylovora*, *Acetobacter diazotrophicus*, and *Bacillus amyloliquefaciens* [137, 155–158]. The efficiency of the enzyme makes them worthy of their use at industrial scale for producing the FOS as well as levan.

Inulosucrase catalyzes formation of β (2–1) linked oligosaccharides and inulin polymer from sucrose. Inulosucrases have been isolated from gram-positive bacteria, such as *S. mutans*, *Leuconostoc citrinum*, *Lactobacillus johnsonii* NCC 533, *Bacillus* sp., *Leuconostoc citreum*

CW28, and *Lactobacillus gasseri* DSM 20604 [114, 135, 153, 158, 159]. Application of FTases is limited by its inhibition in the presence of glucose, a by-product of the fructosyltransferase reaction. Therefore, it becomes crucial to remove glucose from the reaction mixture by its transformation into isomers, employing other enzymes, such as glucose isomerase and glucose oxidase. Glucose oxidase is relatively more effective in obtaining a higher yield of FOS [160, 161]. However, the attempts of enzyme engineering are required to improve the traits of FTases so that its activity should not be compromised in the presence of excess glucose.

### *Synthesis of Fructooligosaccharide from Levan*

Levan is a polymer of D-fructose linked by β (2–6) linkages. Levanases exhibits gylcosyl hydrolase activity leading to FOS production. It has higher substrate specificity for levan, followed by inulin [162, 163]. It has been isolated and characterized from several bacterial sources like *Bacillus subtilis*, *Rhodotorula* sp., *Streptococcus salivarius* KTA-19, *Streptomyces* sp., and *Gluconacetobacter diazotrophicus* SRT4 [102, 162–165]. The endolevanse from *B. subtilis* 168, which is known to exhibit high level specificity towards β (2–6) linkages, has substrate acceptability for bacterial and grass-type levan, but unable to catalyze inulin as substrate [166].

### *Synthesis of Fructooligosaccharide from Inulin*

Inulin is a polymer of fructosyl units that are linked by β (2–1) bonds, with one terminally joined glucose unit through α (1–2) linkage. Inulinases, enzymes of GH 32 family, cleave β (2–1) linkages in inulin, generating FOS. On the basis of cleavage pattern, inulinases are of two types: (i) exoinulinase (EC 3.2.1.80) catalyzes the terminal fructose unit and releases free fructose from inulin and (ii) endoinulinase (EC 3.2.1.7) catalyzes random cleavage of β (2–1) glycosidic bonds within inulin, and thus generating FOS of different chain length, e.g., inulotriose (F3), inulotetrose (F4), and inulopentose (F4). In contrast, exoinulinases are useful in production of high fructose syrup [125, 167]. Inulinases, both extracellular and intracellular, have been reported from a large number of organisms, including bacteria, fungi, and yeast (Tables 4 and 5).

## Substrates and Acceptors Specificity of Enzymes for Synthesis of Fructooligosaccharide

The FOS derived from the same substrate (i.e., sucrose) may differ chemically due to differential action of enzymes, creating different types of glycosidic linkages. The concentration of sucrose in the reaction also affects the enzymatic activity, especially in the cases where both hydrolytic and transferase activities are present in the same enzyme. These enzymes are comfortable with a range of acceptor molecules for the transfer of fructofuranose ring. A number of acceptors have been reported for them such as water, sucrose, raffinose, maltose, maltotriose, arabinose, sorbitol, and xylose [125, 168, 169]. *B. subtilis* NCIMB 11871 FTase catalyzes synthesis of sucrose analogue (Gal-Fru), utilizing the substrates, sucrose, raffinose, and stachyose, by transfer of β-fructofuranosyl moiety to C-1 position of galactopyranoside [170]. Levansucrase from *B. subtilis* NCIMB 11871 catalyzes transferase reaction from β (1–2)-fructosyl to 2-OH of L-sugars (L-glucose, L-rhamnose, L-galactose, L-fucose, and L-xylose) [123]. Synthetic sucrose analogues (Man-Fru, Gal-Fru, Xyl-Fru, and Fuc-Fru) have also been

tested as a substrate, and successful production of a wide range of hetero fructooligosacharides (Gal-(Fru)n-Fru ($n$ > 12) Xyl-(Fru)n-Fru ($n$ = 1–8) were achieved [130]. Recombinant levansucrase from *Clostridium arbusti* SL206 is able to synthesize sufficient amount of raffinose form sucrose and melibiose, a disaccharide of galactose and sucrose linked with α (1–6) glycosidic bond [171].

## Structural and Functional Aspects of Fructansucrases (e.g., Levansucrase)

Levansucrase belongs to GH-68 family of glycosyl hydrolase. The levansucrase protein has three conserved regions with defined functions of (i) a signal peptide and N-terminal stretch with alteration in chain length; (ii) a conserved catalytic domain constituted of ~ 500 amino acids, characteristic of GH-68 family; and (iii) a C-terminal region with varied chain length [47] (Fig. 2).

1. A signal peptide and N-terminal stretch: Mostly levansucrases are secreted extracellular due to the presence of a N-terminal signal peptide of different sizes such as of 37, 32, and 29 amino acids in levansucrase of *Lactobacillus sanfranciscensis* TMW 1.392, *L. mesenteroids*, and *B. subtilis*, respectively [169, 172] (Fig. 2). The cellular mechanism for extracellular secretion of proteins differs in gram-positive and gram-negative bacteria. Levansucrases in gram-positive bacteria (*B. amyloliquefaciens*, *Streptococcus salivarius*, *Lactobacillus reuteri*, *L. mesenteroids*, *B. subtilis*, etc.) are secreted by signal-peptide-dependent mechanism, while gram-negative bacteria (*Gluconacetobacter xylinus*, *Erwinia amylovora*, *Acetobacter xylinum* NCI 1005, and *Zymomonas mobilis*) follow the signal-peptide-independent mechanism [157, 173]. The extracellular secretion of levansucrase in a signal-peptide-dependent pathway is an exception in case of the gram-negative bacterium, *Gluconacetobacter diazotrophicus* [157]. Levansucrase of *Lactobacillus sanfranciscens* contains an unusual direct repeat of DNATSGSTKQESSIAN (16 × 7) adjacent to the signal peptide that does not show homology with other FTases [169]. Further, there is a stretch of hydrophobic amino acid next to the signal peptide, for which no function could be assigned so far; however, its deletion causes an increase in the enzyme activity [174].
2. Catalytic domain: High-resolution crystal structure of levansucrase of *B. subtilis* (in ligand free as well as in substrate bound from at the resolution of 1.5 and 2.1 Å) revealed the presence of five-bladed β-propeller. This kind of fold was firstly observed in GH-43 family α-L-arabinanase A43 (Arb43A) of *Cellvibrio japonicas* [175, 176]. *B. subtilis* levansucrase showed the topology similar to other β-propellers, i.e., five sheets (I–V) folded in a manner forming a "W" shape with four antiparellel β-stands. Like

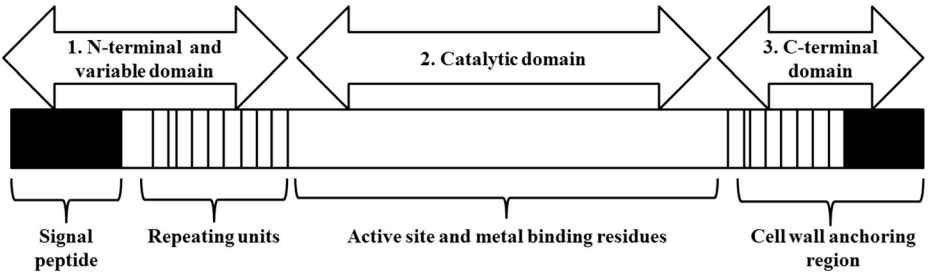


**Fig. 2** A hypothetical diagram showing the arrangement of domains in fructosyltransferase

levansucrase of *B. subtilis*, crystal structure of *G. diazotrophicus* shows a similar kind of five-bladed β-propeller-type structure, though it is a gram-negative bacteria [177]. The highly conserved motifs in the GH 68 family are VWD-86, EWSG-165, RDP-248, DEIER-343, and TYS-412, which contribute in cavity formation of propeller. In GH 32 family, glycoside hydrolase enzymes additionally contain EWSG-165 and RDP-248 as conserved motifs [176].

The acidic amino acid residues, e.g., Asp and Glu, are mostly involved in synthesis of the central cavity in the catalytic domain of FTases and levensucrases. Mutation in Asp247 to Asn led to substantial decrease (75- to 3500-fold) in $K_{cat}$ values, while no significant change was observed in $K_m$ of levansucrase [177]. In *Z. mobilis* levansucrase, substitution of Glu 278 with Asp caused reduction in $K_{cat}$ value by 30-fold, but substitution with histidine led to drastically reduced enzyme activity [136]. Asp194 and Glu 278 are involved in acid base catalysis; carboxylate of Asp acts as nucleophile and attacks on the oxygen linked to C-1 of fructosyl, whereas the carboxyl group of Glu278 acts as a proton donor and consequently cleaves the β (1–2) bond, liberating glucose. Thus, fructose moiety, which is bound as intermediate to the enzyme, is transferred to C-2 or C-6 position of the fructose residue of another sucrose molecule [178]. Mutational studies on *B. subtilis* levansucrase revealed that Asp86 and Glu342 are essential for its catalytic activity. Asp247 is needed at the time of catalysis, but its direct role is not clear. Further, the residues involved in substrate binding and release of the product are not obvious [47]. In levansucrase of *B. subtilis*, Arg331 (His296 in *Z. mobilis*) are found crucial for executing polymerization reaction. The mutants (Arg331Lys, Arg331Ser, and Arg331Leu) in *B. subtilis* were unable to produce levan, but kestose was produced [155].

Generally, metal ions are not essential for the activity of levansucrases; however, presence of $Ca^{2+}$ ion may give a positive impact on its activity. Levansucrase of *S. salivarius* showed 1.5-fold enhancement in the activity upon addition of 1 mM $Ca^{2+}$ in the reaction [136]. The 3D structure analysis of *B. subtilis* levensucrase suggested a possible role of Asp339 residue in interaction with $Ca^{2+}$ [176].

3. C-terminal domain: In FTase, C-terminal domain is involved in substrate specificity and product size [47]. The C-terminal domain contains a cell-wall anchoring motif LPXTG, which helps in the attachment of the enzyme to the cell wall of the host organism. The signature sequence has been identified in levansucrase of *S. salivarius* ATCC 25975 l, inulosucrase, and levansucrase from *L. reuteri* 121 [176, 177].

## Applications of Fructooligosaccharides

FOS is a well-known preferential carbon source for probiotics. It enhances growth of beneficial intestinal microbiota and impedes pathogenic organisms. Apart from bifidogenic effects, the regular and adequate intake of the non-digestible FOS gives beneficial effects in case of the problems associated with gastrointestinal disorders, obesity, diarrhea, osteoporosis, atherosclerotic, cardiovascular, and type 2 diabetes diseases [179]. FOS is recommended to the patients suffering from acute diarrhea, which is a common problem in children. FOS is known to stimulate the absorption of water and electrolyte in the gut mucosa [180, 181]. It has been investigated that the mixture of fructooligosaccharide and galactooligosaccharide is helpful in controlling the symptoms of phenylketonuria in infants [181]. Consumption of FOS reduces

generation of genotoxins and β-glucuronidase enzyme that generate carcinogens in the intestine, and thus regulating incidences of colon cancer [2, 182]. FOS is also useful in controlling inflammatory bowel diseases such as Crohn's disease and ulcerative colitis disease [183]. Diarrhea is generally caused by *Clostridium difficile*. Consumption of FOS and inulin has been shown to lower the colonization of *Clostridium* sp. in the intestine, reducing the risk of diarrhea [4, 184].

In recent days, fructooligosaccharides have been emerged as a prebiotic functional food additive of GRAS (Generally Recognized As Safe) status. Use of inulin improves the quality of bread and skim milk. FOS has laxative property; therefore, nowadays it is included in formulae and food products for infants. Inulin and FOS are used to enhance free fatty acid profile of cheese. The taste profile of FOS is quite similar to sucrose, with ~ 30% less sweetness, higher water retention capacity and no cooling effect. Therefore, it is used in food products as low or no added sugars in formulations such as ice creams, dairy dessert, yogurts, and bakery products [185]. The fermentation of FOS in intestine generates short-chain fatty acids and other organic acids that decrease luminal pH, thereby enhancing the bioavailability of nutritionally important minerals [183, 186]. Increased calcium absorption, as a result of FOS intake, has been demonstrated, which potentially increases bone mineral density [187].

## Future Prospects

Increasing trends in consumption of healthy foods containing non-digestible carbohydrates as health-promoting prebiotic ingredients have generated a huge market for oligosaccharides. This has created a demand of utilization of natural sources containing prebiotic oligosaccharides. As exploitation of natural resources for large-scale production of oligosaccharides is not affordable, the novel technologies for pilot production of these high-value functional biomolecules become crucial. Limited information is available on structural and functional attributes of enzymes useful in oligosaccharide synthesis. Efforts are needed to explore more about the mechanism of action and function of different domains and motifs of these enzymes. Limited knowledge about the relationship between structure and functional property of prebiotics is a barrier in preparation of the best possible mixture of oligosaccharides that can act as a potential prebiotic formulations. Genetic engineering approaches are to be employed for the development of efficient and stable biocatalysts leading to industrial production of prebiotic oligosaccharides. The technology development not only requires exploration of organisms expressing enzymes for oligosaccharide generation, enzyme engineering, and designing of engineered cellular factories for enzyme production and prebiotic molecule biosynthesis, but also the utilization of low-cost abundant biomass and agro-industrial residues for accelerated and economical production of prebiotic and functional molecules. Further, the challenge lies in the economical downstream processing and production of oligosaccharides with an acceptable level of purity.

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**Compliance with Ethical Standards**

**Conflict of Interest** The authors declare that they have no conflict of interest.

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