Review

Fructooligosaccharides (FOS) Production by Microorganisms with Fructosyltransferase Activity

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Abstract: Fructans are fructose-based polymers, defined as fructooligosaccharides (FOS), when they possess a short chain. These molecules are highly appreciated in the food and pharmaceutical international market and have an increasing demand worldwide, mainly for their prebiotic activity and, therefore, for all their health benefits to those who consume them constantly. Thus, new natural or alternative FOS production systems of industrial scale are needed. In this regard, microorganisms (prokaryotes and eukaryotes) have the potential to produce them through a wide and diverse number of enzymes with fructosyltransferase activity, which add a fructosyl group to sucrose or FOS molecules to elongate their chain. Microbial fructosyltransferases are preferred in the industry because of their high FOS production yields. Some of these enzymes include levansucrases, inulosucrases, and β-fructofuranosidases obtained and used through biotechnological tools to enhance their fructosyltransferase activity. In addition, characterizing new microorganisms with fructosyltransferase activity and modifying them could help to increase the production of FOS with a specific degree of polymerization and reduce the FOS production time, thus easing FOS obtention. Therefore, the aim of this review is to compile, discuss, and propose new perspectives about the microbial potential for FOS production through enzymes with fructosyltransferase activity and describe the modulation of FOS production yields by exogenous stimuli and endogenous modifications.

Keywords: FOS; fructosyltransferase; microorganisms; production

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1. Introduction

Fructans are fructose-based polymers usually linked by $\beta(2\rightarrow1)$ and/or $\beta(2\rightarrow6)$ moieties, which possess, if any, a D-glucose unit in the molecule [1]. These carbohydrates are classified based on their linkage type, degree of polymerization (DP), and biological source [2,3]. Thus, according to their DP, fructans are generally classified as fructooligosaccharides (FOS, DP3–DP12) and high polymerization degree fructans (HPD, DP > 12). Both classes possess linear and branched structures [4]. However, independent of their structures, fructans are not metabolized in the human upper gastrointestinal tract. This is due to the lack of enzymes to break β-linkages. On the other hand, the microbiota in the intestinal tract possesses the enzymes needed to catabolize such carbohydrates [5]. Thus, fructans are considered functional food ingredients because they stimulate positive biochemical processes in humans after bacterial fermentation [6]. In this context, fructans can regulate the composition of the human gastrointestinal microbiota and favor the proliferation of good bacteria, for instance, *Lactobacillus* and *Bifidobacterium*. The fermentation of fructans by microbiota in the large intestine results in the production of short-chain fatty acids (SCFAs), mainly acetic, propionic, and butyric acids. These metabolites have been reported to have several benefits to human health, for instance, their anti-cancer activity [7,8]. In addition,

these molecules contribute to gut integrity by regulating the luminal pH and mucus production, providing energy for epithelial cells, and regulating specific mechanisms related to appetite, energy balance, glucose homeostasis, and immunomodulation [9]. Therefore, the integration of fructans into the diet is widely suggested; however, exceeding 20 g/day is not recommended [10]. Some of the health benefits experienced by fructan consumers are constipation relief, decrease in blood glucose levels, control of obesity and diabetes, improvement of mineral absorption, stimulation of the immune system, reduction in oxidative stress, brain protection, and reduction in the risk of developing colon cancer [11–15].

Currently, there is an increase in the consumption of healthy products, which is the reason for the increase in prebiotics demand [16,17]. Among different DP fructans, FOS are considered as prebiotics. Other established prebiotics in the market include galactooligosaccharides (GOS), while isomaltooligosaccharides (IMOS) and xylooligosaccharides (XOS) are considered as emerging prebiotics [18,19]. Several reports indicate that specifically, FOS can decrease total cholesterol and serum lipids, including triglycerides and phospholipids, increase beneficial intestinal microflora, increase the absorption of calcium, magnesium, and iron, inhibit the growth or proliferation of pathogens, positively stimulate the immune system, increase the IgA secretion, decrease proinflammatory cytokines, and exert antioxidant properties [17,20–23]. In addition, FOS improves the organoleptic properties of foods and their texture, taste, and shelf-life. Additionally, these molecules can be used as low-calorie carbohydrate substitutes [24]. Thus, the physical, chemical, technological, and functional properties of FOS are highly appreciated by the food and pharmaceutical industry. The Transparency Market Research [25] estimated the prebiotics market in 2018 at USD 4.5 billion, while the Grand View Research Inc. estimated USD 6.05 billion in 2021 [26]. Based on current tendencies, the value of prebiotics will be around USD 7.11 billion in 2024 [27].

Nevertheless, due to their large demand in the market, new FOS sources and/or biotechnological approaches for their massive production are needed. For instance, the production and consumption of FOS in the United States of America in 2021 was valued at USD 2.76 billion and is projected to reach USD 6.79 billion in 2030, growing at a compound annual growth rate (CAGR) of 10.5% from 2023 to 2030 [28]. These days, FOS are under the loop of functional food research as one of the most promising ingredients. In this context, reports of some microorganisms with the capability of producing FOS through their own enzymes have been increasing in recent years. Such microorganisms synthesize fructans through diverse mechanisms, including transfructosylation and carbohydrate hydrolysis. This fact has positioned microorganism-based-FOS production in the limelight of scientific scrutiny and biotechnology development. Therefore, the aim of this review is to compile, discuss, and propose new perspectives on the microbial potential for FOS production through enzymes with fructosyltransferase activity and the modulation of yields by exogenous stimuli and endogenous modifications.

2. Fructan Diversity

Depending on the fructan source, there are variations in their chemical arrangements, which occur even among species in the same genus. Such structural differences might be dictated by environmental factors [12]. Thus far, based on structural features, fructans are categorized as inulin, levan, graminan, neo-linear fructans, and highly branched neofructans, so-called agavins. Inulin is a linear fructose polymer with $\beta(2\rightarrow1)$ glycosidic linkages with a glucose unit in a reducing terminal position. When there is no glucose in fructans, they are called *F*-series. Inulins are produced by dicotyledonous species belonging to the Asteraceae family. Some of the specimens of this family producing inulin include chicory (*Cichorium intybus* L.), Jerusalem artichoke (*Helianthus* L.), artichoke (*Cynara cardunculos* L.), dahlia (*Dahlia pinnata* Cav.), and yacon tuber (*Smallanthus conatus* (Spreng.) H.Rob.) [29]. Levan is also a fructose-based polysaccharide. Different from inulin, it predominantly possesses $\beta(2\rightarrow6)$ moieties between fructose units. They are mainly found in some grasses, for example, *Dactylis glomerata* L., *Phleum pratense* L. and *Puccinellia*

rupestris (With.) Fernald and Weath [30]. Graminans are defined as mixed-branched fructan polymers; that is, their structures possess both $\beta(2{\rightarrow}1)$ and $\beta(2{\rightarrow}6)$ linkages, and glucose is always located as a terminal residue. Graminans are common components found in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and some members of the Asparagales such as *Arthropodium cirrhatum* (G. Forst.) R.Br., *Astelia banksii* A. Cunn., and *Bulbinella hookeri* (Colenso ex Hook.) Cheeseman [30–32]. Neo-series are built up from neokestose. Thus, they always possess an internal glucose unit in their structures, and there are neo-inulin and neo-levan series [30]. These special series of fructans have been reported in bulbs of the Liliaceae family, for example, onion (*Allium cepa* L.), garlic (*Allium sativum* L.), asparagus (*Asparagus officinalis* L.), and perennial ryegrass (*Lolium perenne* L.) [33–36]. A relatively recently discovered type of neo-fructan called agavin, which are defined as highly branched neo-fructans commonly found in agave species [37–40]. Finally, the most recently described fructan possesses a novel $\beta(2{\rightarrow}3)$ linkage and high molecular weight (1.70×10^3 kDa), and it was reported in the *Radix Codonopsis*. The structure of this fructan was confirmed by one dimension and bidimensional nuclear magnetic resonance, but the new class of fructan has not been placed in any of the abovementioned categories [41]. Table 1 shows a general fructan classification.

Table 1. Fructans categories.

Fructan Type	Linkage	Example	Reference
Inulin	linear, $\beta(2{\rightarrow}1)$	Chicory (*Cichorium intybus* L.), Jerusalem artichoke (*Helianthus* L.), Artichoke (*Cynara cardunculus* L.), Dahlia (*Dahlia pinnata* Cav.), Yacon tuber (*Smallanthus connatus* (Spreng.) H.Rob.)	[29]
Levan	linear, $\beta(2{\rightarrow}6)$	*Dactylis glomerata* L., *Phleum pratense* L., *Puccinellia rupestris* (With.) Fernald and Weath.	[30]
Graminan	$\beta(2{\rightarrow}1)$ and $\beta(2{\rightarrow}6)$ and branched	*Triticum aestivum* L., *Hordeum vulgare* L., *Arthropodium cirrhatum* (G. Forst.) R.Br., *Astelia banksii* A. Cunn., *Bulbinella hookeri* (Colenso ex Hook.) Cheeseman	[30–32]
neo-Fructan (neo-inulin)	neokestose, predominantly $\beta(2{\rightarrow}1)$	Onion (*Allium cepa* L.), Garlic (*Allium sativum* L.), Asparagus (*Asparagus officinalis* L.)	[33–35]
neo-Fructan (neo-levan)	neokestose, predominantly $\beta(2{\rightarrow}6)$	Perennial ryegrass (*Lolium perenne* L.)	[36]
neo-Fructan (agavins)	neokestose, $\beta(2{\rightarrow}1)$ and $\beta(2{\rightarrow}6)$, highly branched	*Agave tequilana Weber var. Azul*, *Agave angustifolia*, *Agave potatorum*	[37–40]
No name	$\beta(2{\rightarrow}3)$	*Radix Codonopsis*	[41]

Fructans play a role as a carbohydrate reservoir in several monocots and 15% of flowering plants [42]. The final chemical structure of plant fructans is determined by several enzymes present in vacuoles [43]. In this regard, sucrose is the substrate for the enzyme sucrose:sucrose 1-fructosyltransferase (1-SST, EC 2.4.1.99), which initiates the fructan synthesis by transferring a fructofuranosyl residue from one sucrose to another sucrose molecule, resulting in the trisaccharide 1-kestose. Subsequently, 1-kestose is used by the enzyme fructan:fructan 1-fructosyltransferase (1-FFT, EC 2.4.1.100), which elongates the fructosyl chain [44]. Fructan synthesis of the neo-inulin series is carried out by fructan:fructan 6G-fructosyltransferase (6G-FFT, EC 2.4.1.243). The formation and extension of $\beta(2{\rightarrow}6)$ moieties in graminans is produced by the sucrose:fructan 6-fructosyltransferase (6-SFT, EC 2.4.1.10). Fructans produced by plants reach a DP ranging between three to a few hundred fructose units [30].

3. Enzymes Involved in Fructooligosaccharides Production

The production of FOS at a commercial scale is achieved by chemical or enzymatic hydrolysis mediated by inulin or sucrose transfructosylation [45–47]. Nevertheless, the chemical synthesis of FOS is a laborious and multi-step approach. Additionally, it does not guarantee the obtention of pure products (DP), but a mixture of them varying their ratios. Because of this, plus a low production yield, it is common to find commercial products with purity ranging between 55 and 99% and other sugar traces [48]. Therefore, the use of biocatalyst enzymes obtained from microorganisms is preferred over chemical synthesis to obtain FOS for human consumption. In this regard, the chemical hydrolysis of inulin makes usage of mineral acids, which are toxic chemicals such as hydrochloric acid, sulfuric acid, and phosphoric acid, which, in addition lack DP-specificity (more than one DP is generated) for the production of FOS [49–51], and their remnants can damage the consumer. Some examples of other contaminants of the chemical synthesis of FOS include colorants and flavoring in the inulin hydrolysate as well as excessive ash content [49,52,53]. In addition, the production of elongation and fermentation inhibitors, such as 5-hydroxymethylfurfural (HMF), can substantially affect the fermenting organisms under high concentrations [51]. It has been reported that in decreasing order, the HMF production during chemical hydrolysis was improved by the addition of phosphoric acid, hydrochloric acid, and sulfuric acid [52]. Moreover, chemical hydrolysis generates by-products such as difructose anhydrides [51,54].

Commonly, the enzymes used in the industry are produced by microorganisms, mainly because of their high purity and enzyme activity, stability, less by-product generation, and profitable manufacturing [55]. In 2015, the biocatalytic synthesis of FOS at an industrial scale generated a high production yield of these carbohydrates, that is, an annual production of 134,000 t [20]. For this approach, two general production stages are required: (1) enzyme production and (2) carbohydrate biotransformation under controlled conditions [17]. Additionally, the production of FOS depends on the bioreactor conditions, including temperature, pH, speed agitation, and oxygen flow [56]. Depicting the high production yields, it is also common for this approach to obtain FOS mixtures with different DP ranges [20]. However, the production of these carbohydrates could be mediated by enzymes with inulinase activity or by fructosyltransferase activity [48,57].

In detail, inulinases are enzymes that hydrolyze inulin into free fructose [58], thus called β-D-(2→1) fructan fructohydrolases with glycosidase/hydrolase activity. These enzymes can be classified as exoinulinases (E.C. 3.2.1.80) and endoinulinases (E.C. 3.2.1.7). For instance, exoinulinases catalyze the remotion of a fructose unit from the fructan chain by hydrolysis up to the fructose part of the initial sucrose molecule. Conversely, endoinulinases catalyze the hydrolysis of internal moieties of the inulin chain. Some products generated by these enzymes are inulotriose, inulotetrose, and inulopentose. Another typical feature of inulinases is that they do not possess invertase activity to hydrolyze the sucrose residue. Partial hydrolysis of inulin by inulinases generates FOS, which generally lacks the glucose-terminal residue, and they are exclusive of the β(2→1) type, with a polymerization degree greater than fructose units. Conversely, transfructosylation is a type of glycosidation that adds a fructosyl group to sucrose or FOS molecules. Some of the enzymes with this potential are β-fructofuranosidases (FFase, E.C. 3.2.1.26) and fructosyltransferases (FTase, E.C. 2.4.1.9) [20,56,59–61].

FOS synthesis by transfructosylation produces mainly trisaccharides and tetrasaccharides. An advantage of these kinds of bioprocesses is that their chemical structure could be determined by the specificity of the enzyme product, making the production of FOS by this route more versatile [62]. The enzymes used can be produced by fermentation in solid or submerged medium through bacteria or fungi. Whole cells and free or immobilized enzymes can be used [49]. Using whole cells in bioprocesses reduces them to a single step, which is an economical strategy because no purification process is needed. However, the presence of microorganisms also requires purification. In addition, they present low yields, non-sugar compound elimination is required, and nutrients and buffer solutions are also required [47]. It is common that during fermentation, FOS with different chemi-

cal structures are produced, that is, a mixture of oligosaccharides with different DP [20]. Nevertheless, this could be reduced by using enzymes and specific microorganisms. In this context, some parameters of the bioprocesses are determinants for FOS production [63], for example, temperature, pH, agitation speed, oxygen flow, and the culturing medium composition [56,63]. The worldwide increase in the demand for FOS requires new substrates and processes to reduce costs, as well as addressing the global problem of excessive generation of agro-industrial waste on the planet [17]. In Mexico, agave juice is an important source of fructans, which have prebiotic properties [64,65]. This juice is employed to produce distilled beverages (tequila and mezcal) and non-distilled beverages (pulque). In the case of pulque, the product is quite a cheap beverage (0.26–0.43 USD/L). Thus, the agave juice of these species is underexploited for commercial purposes. In this sense, agave species used for pulque production could be explored as a substrate to produce FOS by microorganisms, which could make use of the FOS present in the juice as precursors for the synthesis of new fructan molecules. In this sense, the prebiotics and probiotics industry require mathematical models to characterize the parameters that dictate the enzymatic synthesis of FOS. Thus, finding the proper microorganism for FOS production and the most efficient enzymes would lead to the production of FOS through catalytic biosynthesis at the industrial level. This information would serve as the basis for optimizing the FOS production process using computational tools [66].

4. General Fructosyltransferase Activity

Enzymes considered responsible for FOS synthesis are called fructosyltransferases, more specifically, inulosucrase (EC 2.4.1.9) for inulin synthesis and levansucrases (EC 2.4.1.10) for levan synthesis [67]. As previously mentioned, FFases and FTases catalyze the transfructosylation of a fructose unit to a sucrose or FOS molecule, which results in the increase in fructans DP [68]. In this context, FTases have been reported in more than 36,000 plant species and microorganisms, such as filamentous fungi, bacteria, and yeasts [48]. Nevertheless, in plants, the synthesis of fructans is the result of a series of diverse enzymes, while in microorganisms, fructans result from the action of a single enzyme [69]. Moreover, the FTase properties change depending on the microorganism and culture media; for example, the carbon source can play a determining role as an inductor [60]. The reaction mechanism of FTases depends on the biological enzyme origin. Thus, enzymes such as FTases and FFases (also known as invertases) are enzymes commonly used in the industrial production of FOS [3,61]. Depicting how they are used with the same purpose, these enzymes differ in their protein subunit structures, molecular weight, glycosylation degree capability, chemical susceptibility, and substrate specificity [61].

4.1. Microbial Fructosyltransferase Activity

Microorganisms are distributed in three biological domains: bacteria, archaea, and eukarya. Studies in microorganisms describe two enzymes with fructosyltransferase activity—FTases and FFases—reported as FOS-producing enzymes [70]. However, differences between them are not yet well established, which makes it difficult to differentiate between each other [71]. Wild microorganisms in which fructosyltransferase activity has been reported include bacteria, fungi, and yeasts. Nevertheless, new studies have shown potential fructosyltransferase activity in specimens of archaea [72]. FOS produced by these microorganisms possess $\beta(2\rightarrow6)$ (levan-type) or $\beta(2\rightarrow1)$ (inulin-type) linkages depending on the enzyme acting in their biosynthesis, even varying according to specific taxons [43]. Depicting the fact that plants and microorganisms can produce $\beta(2\rightarrow6)$ or $\beta(2\rightarrow1)$ linkages, microbial fructans are characterized by DP larger than 20,000 fructosyl units [73]. In addition, microbial systems are more efficient than vegetal systems for FOS production since microbes can produce these molecules with a single multifunctional enzyme [74]. In microbes, the FFase first catalyzes the hydrolysis of sucrose, and subsequently, it can perform fructosyltransferase activity, depending on the cellular conditions and microbial

species. Thus, the activities of the enzymes hydrolase (Uh) and fructosyltransferase (Ut) and the Ut/Uh activity ratio will vary according to such variables [47].

FTases and FFases are a large group of enzymes with great biochemical importance that are responsible for the synthesis and degradation of fructans, respectively. The study and analyses of their amino acids show strong similarity at the biochemical and molecular level between FFases and vegetal FTases [75]. FTases and FFases belong to the enzyme family of the GH32 and GH68 glycosyl hydrolases, and both integrate the superfamily denominated the GH-J clan. These enzymes are divided into families based on their amino acid sequence similarities so far, with a total of 135 GH families. Amino acid sequence analyses have shown that GH32 and GH68 are homologous and possess conserved regions shared with families GH43 and GH62 [76]. The family GH32 includes diverse types of enzymes such as β-fructofuranosidases, cell wall invertases (CW-INV), vacuolar invertases (V-INV), inulinases (endo- and exo-inulinases), levanases (endo- and exo-levanases), fructan exohydrolases (FEH: 1-FEH (EC 3.2.1.153), 6-FEH (EC 3.2.1.154), and fructosyltransferases (FTases: 1-SST, 1-FFT, 6-SST, 6-SFT, 6G-FFT, levansucrases) [32,43]. The previous ones can be found in bacteria, fungi, and plants [43]. On the other hand, the family GH68 is less diverse and, so far, only includes β-fructofuranosidases, levansucrases, and bacterial inulosucrases. In the case of microbial enzymes with fructosyltransferase activity, they are classified based on the linkage type they form and their enzymatic properties. A general classification for the enzymes with fructosyltransferase activity studied in microorganisms is shown in Figure 1.

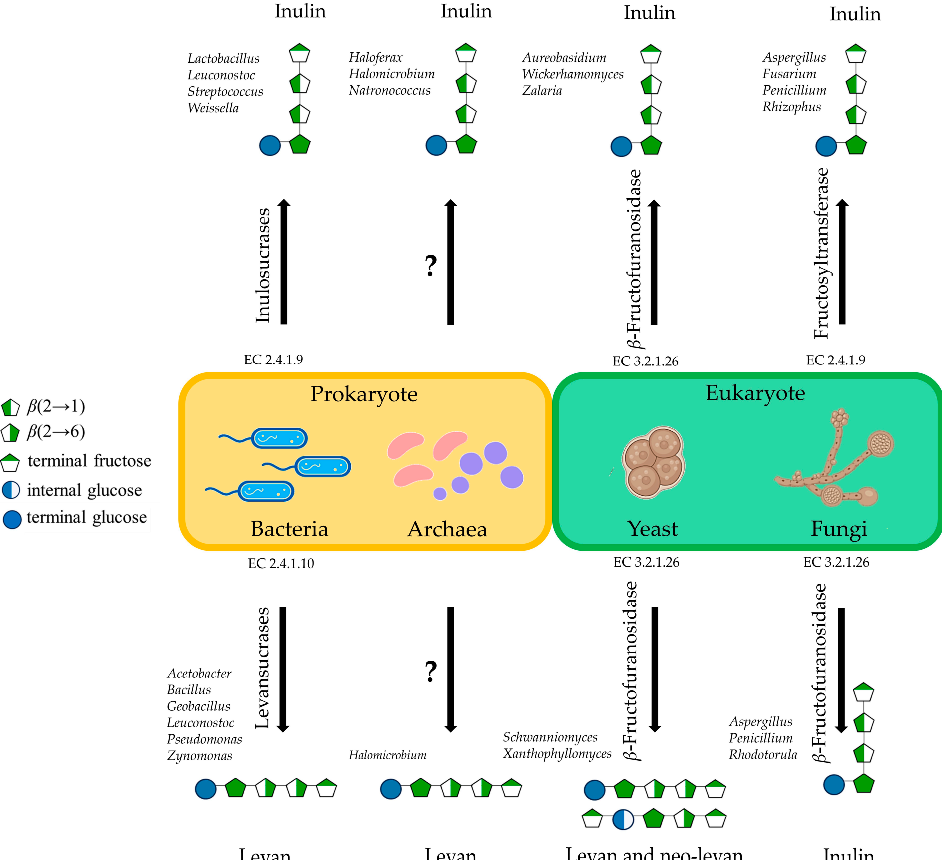

Figure 1. Microbial enzymes with fructosyltransferase activity present in archaea, bacteria, filamentous fungi, and yeast [46,61,72,77–97], and chemical structures were drawn following López and Salomé-Abarca nomenclature [98]. The arrows indicate the type of fructan produced by bacteria, archaea, yeast, or fungi. The question mark indicates that no enzyme identification has been performed for the reported fructan molecule.

4.1.1. Fructosyltransferase Activity in Bacteria

Fructosyltransferases are classified as levansucrases when they synthesize levan $\beta(2\rightarrow6)$ or inulosucrases when they synthesize inulin $\beta(2\rightarrow1)$. It is common that levan molecular weight varies from 10^5 to 10^7 Da, while inulin varies between a few hundred to a few thousand Da [99]. In bacteria, levansucrases have been widely studied, while inulosucrases remain scarcely explored [67]. Levansucrases synthesize FOS with the formula GFn within a DP3–DP10). These molecules are elongated by sequential transfructosylations of fructose units to a sucrose molecule, producing 6-kestose as the smallest levan, along with other 6*F*-series [100,101]. Additionally, some levansucrases can produce the FOS of the 1*F* (1-kestose) and the 6*G* series (neo-kestose). Levansucrases also display hydrolytic activity, which leads to the release of free fructose, which is used by these enzymes to produce levan oligomers such as levanbiose and levantriosa, among others [102].

Bacteria commonly produce FOS-levan and HPD-levan as exopolysaccharides (EPS) secreted as components for the formation of extracellular biofilms. This biofilm protects the bacteria from environmental factors such as temperature, pH, antibiotics, and host immune defenses [103]. Among those factors, the concentration of sucrose in the growing medium is a determining factor to produce levan [104]. That is, by increasing the sucrose content in the growing media, levan production is increased, too. Nonetheless, a saturation of this disaccharide in the medium might result in a decrease in levan production. This could be caused by an increase in the osmotic pressure and high medium viscosity, which might result in a cellular explosion and low enzyme diffusion.

Structurally, levan produced by bacteria possess mainly $\beta(2\rightarrow6)$ linkages and some $\beta(2\rightarrow1)$ branches [105]. The first reaction for levan synthesis is the formation of 6-kestose by using two sucrose molecules as building blocks. One sucrose molecule acts as a fructosyl donor, and the other as an acceptor. Subsequently, 6-kestose is elongated through the addition of fructofuranosyl residues by transfructosylation reactions [106]. The most studied bacterial genera with transfructosylation activity are *Acetobacter*, *Bacillus*, *Geobacillus*, *Lactobacillus*, *Leuconostoc*, *Pseudomonas*, *Zynomonas*, *Erwinia*, *Gluconobacter*, *Halomonas*, and *Microbacterium* [107,108]. Specific wild bacteria species that produce levan include *Bacillus subtilis* (Natto), *Bacillus licheniformis* ANT 179, *Bacillus licheniformis* BK AG21, *Brachybacterium* sp. CH–KOV3, *Geobacillus stearothermophilus*, *Halomonas smyrnensis* AAD6T, *Lactobacillus reuteri* strain 121, *Paenibacillus polymyxa* EJS-3, *Streptococcus salivarius* SS2, and *Zymomonas mobilis* [83,101,109–116].

Furthermore, levan produced by Gram-negative and Gram-positive bacteria are well differentiated by their DP range. For instance, Gram-positive bacteria synthesize levan with molecular weights ranging between 1×10^4 and 1×10^7 Da, while Gram-negative levan present molecular weights even greater than 1×10^8 Da [117]. Taken as an example, levan produced by *B. licheniformis,* a Gram-positive bacterium, reach a DP up to 5.82×10^6 Da [118], while the Gram-negative *Brenneria* sp. produce levan molecules around 1.41×10^8 Da [119].

On the other hand, inulosucrase enzymes have been identified only in a few Gram-positive bacteria [99], most of them lactic acid bacteria [77,120]. Among them, the genus *Streptococcus* is one of the most studied [79,121]. Rosell and Birkhed (1974) reported an inulin produced by *Streptococcus mutans* [122], a strain related to dental caries [123]. Some differences can be stated between plant and microbial inulins, and they rely mainly on their molecular weight differences. Plant inulin is not heavier than 10^4 Da, while microbial inulin is usually heavier than 10^6 Da [124]. However, inulosucrases have not been employed at an industrial production scale because their FOS production yield in wild bacteria is rather low [74]. Thus, most of these enzyme genes are expressed in other microorganisms. For instance, a gene encoding an inulosucrase from a *Lactobacillus reuteri* strain was successfully overexpressed in *E. coli* [67,125]. Other important conditions affecting the production of FOS by microorganisms can be seen in Table 2.

Table 2. Culturing conditions and fructans production by bacteria.

Bacteria		Enzyme				Culturing Conditions				Product			Reference
		Name	Molecular Weight	Enzymatic Activity	Units	Culture	Component	g/L	Conditions	Name	g/L	$Y_{P/S}$ (%)	
Gram-negative	*Bacillus subtilis* (Natto) CCT7712	Levansucrase	n.d.	23.9	U/mL	Cells	Sucrose Yeast extract KH_2PO_4 $(NH_4)_2SO_4$ $MgSO_4$•$7H_2O$ $MnSO_4$ Ammonium citrate	3502 1 3 0.6 0.2 0.25	Agitation = 50 rpm pH = 6 T = 35 °C t = 36 h	Levan FOS (nystose)	63.6 41.3	18.1 11.8	[126]
	Bacillus subtilis (Natto) CCT7712	Levansucrase	n.d.	60	Mmol/mL	Cells	Sucrose Yeast extract KH_2PO_4 $(NH_4)_2SO_4$ $MgSO_4$•$7H_2O$ $MnSO_4$ Dibasic ammonium citrate ($(NH_4)_2HC_6H_5O_7$)	400 2 1 3 0.6 0.2 0.25	Agitation = 150 rpm pH = 7.7 T = 35 °C t = 48 h	Levan FOS (nystose)	192.41 173.6	48.1 43.4	[63]
	Bacillus methylotrophicus	Levansucrase	n.d.	n.d.	n.d.	Cells	Sucrose Yeast extract KH_2PO_4 Peptone	20 10 4.5 4.5	Agitation = 180 rpm pH = 7.5 T = 30 °C t = 24 h	n.d.	n.d.	n.d.	[127]
			n.d.	6	U/g	Enzyme	Sucrose	300	Agitation = n.s. pH = 6 T = 37 °C t = 16 h	Levan (4–5 kDa)	100	33	
	Bacillus aryabhattai	Levansucrase	n.d.	n.d.	n.d.	Cells	Sucrose GYC media: Yeast extract Glucose $CaCO_3$	250 10 50 5	Agitation = 150 rpm pH = 8 T = 30 °C t = 120 h	Levan (5.317 × 10^7 Da, 5.19% branched) FOS (1-kestose, 6-kestose, neokestose, nystose and others)	26	10.4	[117]
	Paenibacillus polymyxa EJS-3	Levansucrase	n.d.	n.d.	n.d.	Cells	Sucrose Yeast extract KH_2PO_4 $CaCl_2$	188.2 25.8 5 0.34	Agitation = 150 rpm pH = 8 T = 24 °C t = 60 h	Levan	35.26	18.7	[128]

Table 2. *Cont.*

Bacteria		Enzyme				Culturing Conditions				Product			Reference
		Name	Molecular Weight	Enzymatic Activity	Units	Culture	Component	g/L	Conditions	Name	g/L	$Y_{P/S}$ (%)	
Gram-positive	*Zymomonas mobilis*	Levansucrase	n.d.	n.d.	n.d.	Cells	Sucrose Yeast extract KH_2PO_4 $(NH_4)_2SO_4$ $MgSO_4 \bullet 7H_2O$	250 2.5 1 1 0.5	Agitation = n.s. pH = n.s. T = 24 °C t = 24 h	Levan	21.69	8.67	[104]
	Zymomonas mobilis	Levansucrase	n.d.	n.d.	n.d.	Cells	Sucrose Yeast extract KH_2PO_4 $(NH_4)_2SO_4$ $MgSO_4 \bullet 7H_2O$	299.1 1 1 0.5	Agitation = 0 rpm pH = 6 T = 28 °C t = 42.3 h	Levan	40.2	13.44	[129]
	Leuconostoc citreum CW28	Inulosucrase	170	n.d.	n.d.	Cells	Sucrose Yeast extract K_2HPO_4 $MgSO_4$ $CaCl_2$ NaCl $MnSO_4$ $FeSO_4$	20 20 20 0.2 0.05 0.01 0.01 0.01	Agitation = 200 rpm pH = 6.9 T = 30 °C t = 12 h	Inulin	n.d.	n.d.	[78]

n.d. = not determined, n.s. = not specified.

4.1.2. Fructosyltransferase Activity in Archaea

Metagenomic DNA sequencing has elucidated the biosynthetic potential of archaea strains to produce fructans since they possess fructosyltransferase (*ftf*) genes in their genomes [130]. The genes of the GH-J clan have been found to be extremely halophilic Archaea [131]. Thus far, three archaea, *Halomicrobium mukohataei* DSM 12286, *Haloferax prahovense* DSM 18310, and *Natronococcus jeotgali* DSM 18795, isolated from America, Europe, and Asia, respectively, and other four Halomicrobiums (called IBSBa, IBSBb, IBSBc, and IBSBd) from the salt Tuz Lake synthetized different types of fructans. The nuclear magnetic resonance (NMR) analysis showed that *H. mukohataei, H. prahovense, N. jeotgali,* and the strain IBSBa produced fructans like inulin, while strain IBSBb produced levan. These archaea possessed GH32 and GH68 genes [72]. The physiological functions of fructans in halobacteria are a big opportunity to explore how fructans help the archaea in hypersaline environments where sucrose is available.

4.1.3. Fructosyltransferase Activity in Microbial Eukaryote

Eukaryote microbial fructosyltranferase activity has been reported to occur in fungi, which include both filamentous fungi and yeast. Fungi and vegetal FTases, like the rest of the invertases, belong to the 32 families of glycosylhydrolases [43]. Filamentous fungi FTases are evolutionarily closer to plant (eukaryote) β-fructofuranosidases rather than to bacterial ones [76]. A strong biochemical and molecular similarity between vegetal invertases (FFases) and FTases suggests that vegetal FTases evolved from invertases [75]. Differently from plants, fructan synthesis in fungi is generally catalyzed by a single 1-SST, which results in the production of 1-kestose, 1-nystose, and 1-fructosylnystose [3]. Nonetheless, some fungi, such as *Aspergillus japonicus* or *Aspergillus terreus*, produce β-fructofuranosidases (FFases) with fructosyltransferase activity [132,133]. Interestingly, in fungi, the type of activity of these enzymes depends on the sucrose concentration in the medium. For instance, at low sucrose concentrations, the FFases display hydrolytic effects over fructans, while at high sucrose concentrations, the enzymes show transfructosylating activity [134]. Nonetheless, there are some exceptions, such as *Aspergillus niger* AS0023, which possessed both FTases and FFases. Moreover, *A. niger* FTase displayed only transfructosylation activity, while its FFase did not exhibit fructosyltransferase properties [71]. Furthermore, *Aureobasidium pullulans* are considered a source of FTases and FFases. FTases have been reported in several microorganisms, predominantly in filamentous fungi, including *Aureobasidium*, *Aspergillus*, *Fusarium*, *Neurospora*, *Penicillium*, and *Rhizophus* [47,69], particularly in species such as *Aureobasidum pullulans, Neurospora crassa, Fusarium oxysporum, Aspergillus niger,* and *Aspergillus oryzae* [45,60,135]. Conversely, the information about levansucrase enzymes in fungi is still scarce. Now, there is little information about levansucrases in fungi; for example, a levansucrase recently reported in *Aspergillus awamori* EM66 [136]. As in bacteria, the culturing medium conditions also affect the activity of the fungal cells and their enzymes. Some of the factors that cause changes in the fructosyltranferase activity are temperature, pH, agitation, and culturing time [137] (Table 3).

Table 3. Culturing conditions and fructan production by filamentous fungi.

Fungi	Enzyme				Growing Conditions				Product			Reference
	Name	**Molecular Weight**	**Enzymatic Activity**	**Units**	**Culture**	**Component**	**g/L**	**Conditions**	**Name**	**g/L**	**$Y_{P/S}$ (%)**	
Aspergillus oryzae DIA-MF	FTase	n.d.	1347	U/L	Cells	Aguamiel (content of sucrose)	37	Agitation = n.s. pH = 4.5 T = 30 °C t = 48 h	FOS	15.5	41.89	[137]
	FTase	n.d.	1431	U/L	Enzymatic extract	Aguamiel (content of sucrose)	37	Agitation = n.s. pH = 5 T = 30 °C t = 3 h	FOS (kestose)	11	29.72	
Aureobasidium pullulans CCY 27-1-94	FTase	570,000	1310	U/mg	Enzyme	Sucrose	600	Agitation = n.s. pH = 5.5 T = 55 °C t = 8.3 h	FOS kestose nystose	300 150 150	50 25 25	[138]
Aspergillus aculeatus	FTase	n.d.	25.9	U/g	Enzymatic extract	Sucrose	630	Agitation = 1000 rpm pH = 5.6 T = 60 °C t = 36 h	FOS kestose n.s. fructofuranosylnystose	387 240 144 3	61.42 38.09 22.85 0.47	[61,139]
	FTase	135 kDa for dymer	5	U/mL	Enzyme	Sucrose	600	Agitation = n.s. pH = 5.5 T = 60 °C t = 24 h	FOS kestose nystose fructosylnystose	364.2 112.2 213.6 38.4	 60.7 18.7 35.6 6.4	
Cocrophilous fungi (*Aspergillus niger* sp. XOBP48)	FTase	n.d.	n.d.	n.d.	Cells	Sucrose Yeast extract $NaNO_3$ K_2HPO_4 $MgSO_4 \bullet 7H_2O$ $FeSO_4 \bullet 7H_2O$ KCl	30 5 3 1 0.5 0.01 0.5	Agitation = 200 rpm pH = 6.5 T = 28 °C t = 168 h	n.d.	n.d.	n.d.	[140]
	FTase	n.d.	529.5	U/mL	Enzymatic extract	Sucrose	50	Agitation = n.s. pH = 6.5 T = 60 °C t = 0.5 h	kestose nystose	n.d.	n.d.	[140]

n.d. = not determined, n.s. = not specified.

Other low eukaryotes, such as yeasts, have been used as models at cell-level studies for a long time [141]. Currently, the genomes of several yeast species are already described. For instance, yeasts have a genome with around 6000 genes, and filamentous fungi possess around 10,000 genes. In this context, they share a large part of their primary metabolism pathways, but they also possess unique metabolic features, especially when considering fructan synthesis [142]. Filamentous fungi produce FOS by sucrose transfructosylation using FFases and FTases [143]. Conversely, FTases are not reported to naturally occur in yeast. Instead, their enzymes are only β-fructofuranosidases (FFases). In yeast, two mechanisms have been proposed for obtaining different FOS DPs, which are hydrolysis and transfructosylation [68]. Unfortunately, there are only a few reports on fructosyltransferase activity in wild yeast. One of the first studies describing invertase (FFasa) in *S. cerevisae* showed its transfructosylation activity when growing in highly concentrated solutions of sucrose [3M] [144]. Later, a broader screening of yeast was performed to evaluate their transfructosylating activity. The analyses showed *Rhodotorula* sp. (LEB-V10), *Candida* sp. (LEB-I3), *Rhodotorula* sp. (LEB-U5), and *Cryptococcus* sp. (LEB-V2) as promising transfructosylating strains. However, they also possessed high hydrolytic activity [145]. That is, they produced FOS, but they also degraded them. Thus, the search for FOS production strains must be focused on the balance between transfructosylation and hydrolysis of such enzymes [88]. Table 4 shows some of the principal factors evaluated over fructosyltransferase activity of yeast and their yields.

Table 4. Culturing conditions and fructan production by yeast.

Yeast	Enzyme				Growing Conditions				Product			Reference
	Name	Molecular Weight	Enzymatic Activity	Units	Culture	Component	g/L	Conditions	Name	g/L	$Y_{P/S}$ (%)	
Schwanniomyces occidentalis	β-fructofuranosydase (with FTase activity)	85 kDa	0.3	U/mL	Enzyme	Sucrose	600	Agitation = 650 pH = 5.6 T = 50 °C t = 24 h	FOS (6-kestose)	76	0.16	[46]
Saccharomyces cerevisiae SAA-612	β-fructofuranosydase (with FTase activity)	n.d.	17.8	U/mg	Enzymatic extract	Sucrose Yeast extract $(NH_4)_2SO_4$ K_2HPO_4 $MgSO_4$	20 10 1 3.5 0.75	Agitation = n.s. pH = 5.5 T = 40 °C t = 4 h	FOS (n.e)	n.s.	n.d.	[146]
Saccharomyces cerevisiae CAT-1	β-fructofuranosydase (with FTase activity)	n.d	n.d.	n.d	Enzymatic extract	Sucrose	200	Agitation = not pH = 4.5 T = 50 °C t = 2 h	FOS (kestose)	13.3	6.65	[147]
Rhodotorula mucilaginosa	β-fructofuranosydase (with FTase activity)	n.d	n.d.	n.d	Enzymatic extract	Sucrose	200	Agitation = not pH = 4.5 T = 50 °C t = 2 h	FOS (kestose)	12.6	6.30	

n.d. = not determined, n.s. = not specified.

4.2. Improvement and Increase in Fructosyltransferase Activity

As previously mentioned, even if several microorganisms display fructosyltransferase activity, only a few have the potential to be used in massive production schemes, for example, *Aspergillus niger* and *Aureobasidium pullulans* [148–151]. However, there are several microorganisms with industrial operative advantages that might be better for FOS production. Nonetheless, finding good strains that produce the target product with good yields is a challenging and time-consuming task [60]. Thus, the improvement of microbial strains for FOS production is a good time-saving alternative. This might be achieved by genetical modification or by varying chemical and physical conditions in the culturing environment of such microorganisms [70]. One of the most important advantages of strain modifications is the overexpression of the genes involved in the synthesis of the target compound [152]. In this regard, when not genetically modified, positive gene mutations can be achieved by chemical and/or physical stimuli. Some of the most used chemical agents to cause gene mutations include alkylating agents such as ethyl methane sulfonate, intercalating agents such as ethidium bromide, and base analogs. On the other hand, physical modifiers include electromagnetic radiation, for example, gamma rays, X-rays, UV light, and particle radiation [153,154].

In the case of fructosyltransferase, several microbial models have been explored to produce FOS by this enzyme. In this regard, fungi are some of the most studied models for obtaining fungal strains with overexpression of fructosyltranferase activity. For instance, *Aspergillus oryzae* ZT65, cultured on a solid medium at 37 °C for 4 h, was continuously irradiated through 50 s intervals with UV light, which induced a positive mutation in a new strain named *A. oryzae* S719. The new isolate overexpressed the Ftase gene, which resulted in high production of FOS. The strain produced around 586 g of FOS/L in 20 h under bioreactor conditions, which corresponds to an FOS production rate of 29.3 g/L/h [152]. Additionally, this strain was shown to be resistant to high osmotic pressures, 900 g of sucrose/L, which was correlated to its high FOS production yield. Whilst a mutant of *Aspergillus niger* was generated by gamma irradiation (1200 Gy) in a Co-60 irradiator, which resulted in a potentiation of the β-fructofuranosidase activity of the new strain. Interestingly, bran was the substrate for enhancing the FOS production by this fungus, which opened the possibility of using agro-industrial residues for the induction of FFase activity [155]. Nevertheless, the production of metabolites or biomolecules by modified organisms is a much more complicated process, as we briefly describe here, and several limitans might appear during the process. For instance, irradiation approaches are random [154,156], and some of the produced mutations might not be conserved, or they can be naturally repaired by the microorganism [157,158]. Different factors affect the responses and, therefore, the efficiency of the mutagenic treatments; thus, a deep knowledge of the organisms' physiology, the biosynthesis regulation points, and the selection of the mutation agents is needed [159]. Thus, mutagen-dose optimization is required for physical or chemical modifications [156].

In this regard, recombinant DNA technology has allowed the development of strategies to obtain systems with better heterologous protein expression (HEP). This approach consists of the expression of a given gene in a transformed organism, for example, the commercial production of recombinant insulin by *E. coli* and *S. cerevisiae* for therapeutic use in humans [160]. To carry out such a task, we need to know the sequence of the target gene, an expression vector, and a host organism, either prokaryotic or eukaryotic. Moreover, it is fundamental to determine the proper culturing conditions, which could vary between non-modified and modified strains, to assure protein expression. To increase the production of FOS, it is necessary to gather knowledge about the enzymes' structural determinants involved in the fructosyltransferase activity, efficiency, substrate, and product specificity [161]. Thus, the increasing worldwide commercial demand for fructans has led to a search for heterologous expression models for enzymes with fructosyltransferase activity production.

So far, the most structurally characterized enzymes belong to the GH-J clan. The enzymes of these clans possess proteins with an N-terminal five-bladed β-propeller domain present in GH32 and GH68 and a C-terminal domain constituted by two β-sheets, which is only present in GH32 [162]. The secondary structure of microbial GH32 proteins possesses eight conservated domains, the so-called A, B, B1, C, D, E, F, and G domains. Domains A, D, and E are conserved acidic residues in the active site of the enzymes [163]. The sequence analysis of members of the GH32 indicates that three conserved acidic amino acids, so-called Asp-60, Asp-191, and Glu-292, are key structural components in the active site of these enzymes (Figure 2). They are called the catalytic triad, and they are considered the catalytic nucleophile, the transition-state stabilizer, and the general acid/base catalyst, respectively [162,164]. The Asp-60, Asp-191, and Glu-292 residues belong to the three conserved sequences referred to as WMNDPNG or β-fructosidase, RDP, and EC motifs, respectively [161]. Figure 2 shows that Asp-60, Asp-191, and Glu-292 are situated at the first β-strand of blades (I, III, and IV, respectively). They are located at the active site on the bottom of the cavity in the center of the β-propeller domain [164]. Invertases usually possess an intact A motif (WMNDPNG), while FTs display structural alterations in this motif [162].

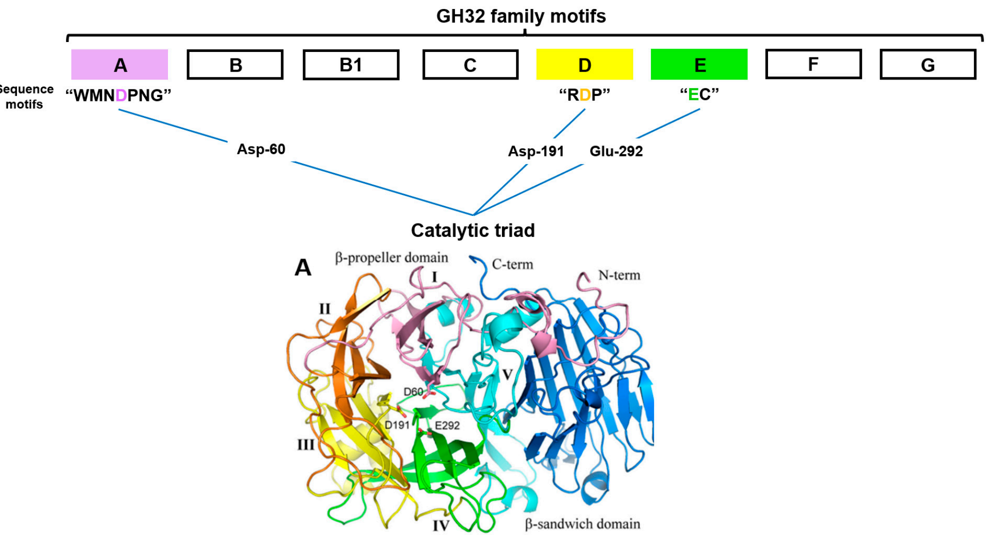

Figure 2. Conserved motifs in enzymes of the GH32 family and their catalytic site of a FTase of *A. japonicus* (taken and modified from [164]). "https://creativecommons.org/licenses/by/4.0/legalcode (accessed on 29 October 2023)".

The GH-J clan, including both hydrolytic and transfructosylating enzymes, acts through a double displacement mechanism. First, a nucleophilic attack occurs in the Asp-60 in the anomeric carbon of the fructosyl moiety of the fructose donor, usually sucrose, forming the intermediate covalent enzyme-fructose (E-F). Subsequently, Glu-292 protonates the leaving group, for example, glucose. In the second stage, Glu-292 deprotonates the acceptor molecule to activate it as a nucleophile. This is achieved by acting over the anomeric carbon of the fructosyl moiety in the E-F complex. The acceptor molecule for invertases may be water, releasing free fructose. For fructosyltransferases, sucrose or fructan will be the acceptor molecule. This results in an increase in the DP of a fructan. Depending on the hydroxyl group of the acceptor molecule, deprotonation by the Glu-292, the type of glycosyl linkages in the FOS molecules will be determined [161,164].

Previous mutagenesis studies of the GH-J clan suggested that the residues in the hydrophobic pocket of the active site, such as Suc2, Trp 19, and Phe 82, are involved in substrate binding. Molecular docking studies describe that in Suc2 gen isolated previously from *S. cerevisiae*, Trp 291 and Asn 223 have an important role: the first one acts as a

platform for binding sucrose as an acceptor molecule, and the second one assists in the orientation of sucrose for the synthesis of $\beta(2\rightarrow6)$ moieties [161].

As discussed earlier, the GH32 and GH68 families have the feature of a five-bladed β-propeller fold as a catalytic core [76], where the active site residues are sited inside of a cavity. Crystal structure analyses of the enzymes with fructosyltransferase activity in microorganisms show some differences. For example, the FTase of Gram-negative bacteria acts as an extracellular enzyme, so they contain an N-terminal signal sequence that targets these enzymes for secretion. In addition, the C-terminal domain of FTase of Gram-positive bacteria contains the motif LPXTG (Figure 3), which is related to cell-wall anchoring [165,166]. Some reports described that levansucrases from Gram-positive bacteria produce polymers, and those from Gram-negative bacteria produce FOS [167,168]. In addition, the length of the domains could change. In fact, the function of the N-terminal domain is not clear, and the C-terminal glucan binding domain is correlated with the structure of the synthesized glucan [166].

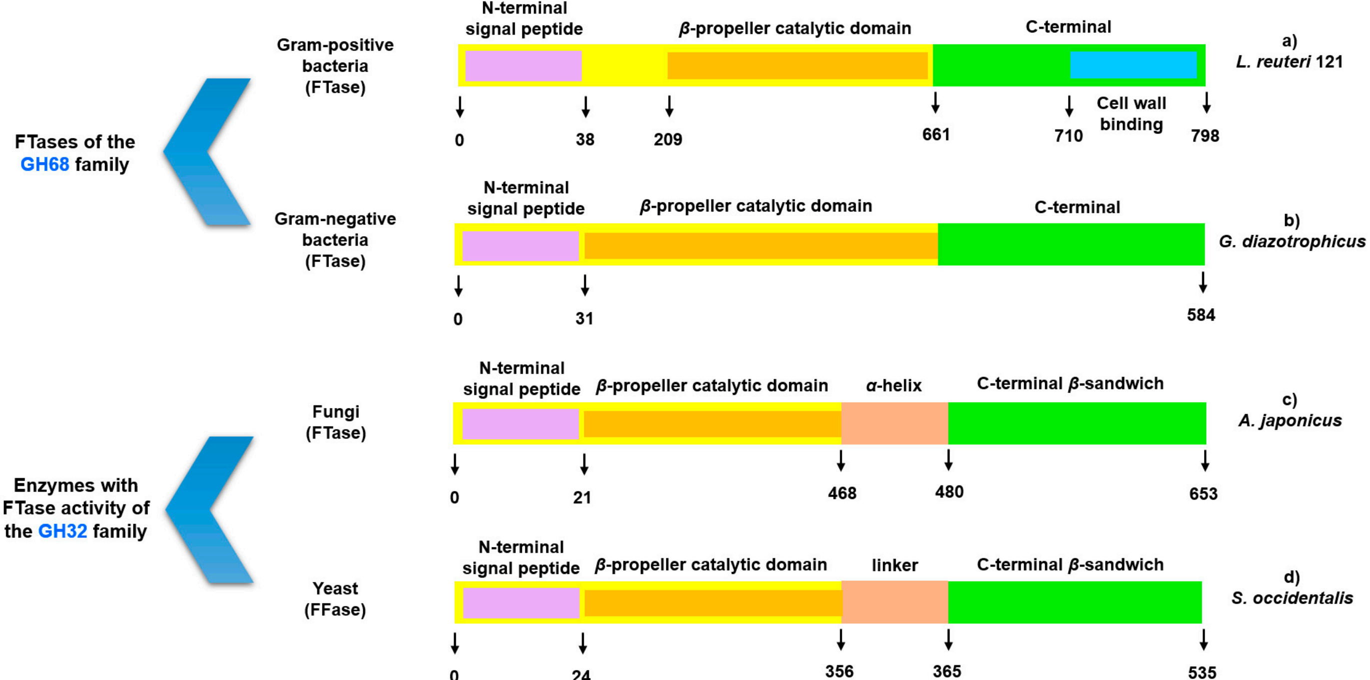

Figure 3. Graphical representation of the structural organization of enzymes with fructosyltransferase activity. Each letter indicates an amino acid sequence with different length coming from diverse microorganisms, the colors indicate distinct regions, and the black arrows the residual amino acids number. Data was compiled from (**a**) [166] and (**b**–**d**) [164,169,170]). "https://creativecommons.org/licenses/by/4.0/legalcode (accessed on 29 October 2023)".

This information is necessary to improve enzymes with fructoyltransferase activity. Thus, a deeper structural characterization and understanding of the function in the fructan synthesis of the cell wall binding, present only in Gram-positive bacteria, is an interesting point to be potentially exploded in the biotechnological approach of such microorganisms. For instance, is this domain related to the differences in the fructan size produced by Gram-positive and Gram-negative bacteria? If yes, can we manipulate it to obtain specific-DP fructans or defined DP ranges of fructans? Moreover, is this domain correlated to differences in the fructan yield production between Gram-positive and Gram-negative bacteria? If so, can we also use it to increase fructan production by microorganisms?

4.3. From Genetic Engineering to Synthetic Biology to Produce fructooligosaccharides in Microorganisms

Genetic engineering is the modification of DNA sequences by molecular biology technology [171]. In this sense, several types of genetic modifications can be made to the genome. For instance, DNA sequence deletions (knockouts), DNA sequence insertions (knockings), and replacements of DNA sequences with exogenous sequences (DNA replacements) [171]. In the case of fructosyltransferases, deciphering the structural features of these proteins correlated with their activity will contribute to designing reliable mutations for improving their catalytic activity or relieving product inhibition [172].

After the development of genetic engineering and applied molecular biology, metabolic engineering appeared in the 1990s [173]. The aim of this area is to improve cellular activities by manipulating enzymes and the transport and regulatory functions of the cell through recombinant DNA technology. Cell activity occurred a long time ago, and metabolic networks evolved in nature. However, most of them are not optimal for important practical applications, which is the reason why metabolic engineering enhances the properties of biological processes by genetic modifications [174]. The introduction of heterologous genes and regulatory elements separates metabolic engineering from traditional genetic approaches to improve a strain [175]. This is focused on metabolic pathways (native or not) for microbial synthesis of bioproducts, which requires design, construction, and optimization [173]. Metabolic engineering represents a powerful tool for approximating the industrial fermentation processes to optimization through the introduction of directed genetic changes using recombinant DNA technology.

Synthetic biology is defined as the development of a rigorous engineering discipline to create, control, and program cellular behavior. The main aim of this field is the application of cell programming to produce specific products, including metabolites, proteins, and enzymes. Synthetic biology involves the use of biomimetic chemistry, in which organic synthesis is used to create artificial molecules through certain enzymes [176]. Thus, it can be stated that synthetic biology requires biological components, in this case cells, to reproduce specific exogenous artificial mechanisms [176]. For instance, the production of some important terpenoids was enhanced by inserting an alternative mevalonate pathway from *S. cerevisiae* in *E. coli* [177]. However, so far, there are no synthetic biology approaches developed for the industrial-scale production of FOS.

In this context, microorganisms provide a variety of bioactive natural products (NP) [178] used by different industrial sectors, which have recently been approached by synthetic biology. Biosynthetic gene clusters (BGCs), defined as physically clustered groups of two or more genes in a specific genome, encode a biosynthetic pathway to produce a specialized metabolite [179]. These genes are involved in the biosynthesis, regulation, resistance, and transport of metabolites, which are codified in a continuous region of the genome. Microbial genomes encode numerous BGCs that may produce natural products with diverse applications in medicine, agriculture, environment, and materials science [178]. The last decades have shown an extraordinary development of high-throughput sequencing and amplicon sequencing approaches targeting the 16S rRNA gene for describing the microbiome composition and diversity. Nonetheless, these approaches will be gradually replaced using metagenome-assembled genomes (MAGs), which allow the mapping and unraveling of the metabolism and ecology of microorganisms [180]. Along with this, the use of genome sequencing and bioinformatics have propelled the heterologous expression of BGCs for NP production. However, this approach had been limited by the dependency of the expression of BGCs on the physiology of a few available host chassis [178]. It is worth mentioning that in synthetic biology, the term chassis is used to refer to an organism that serves as a host for genetic components. In addition, the chassis microorganisms allow the proper expression and function of such genetic components by providing transcription and translation factors [181]. *Escherichia coli* is considered the most employed microorganism in synthetic biology, for example, its use in the production of isopentenol trough the redirection metabolic by Multiplex CRISPRi-Mediated Repression [182]. However, the

genetic expression and regulation of synthetic circuits are highly host-specific; the use of the correct chassis requires an available toolkit of computational design programs, genetic parts, regulatory elements such as promoters, ribosomal binding sites (RBS), and stop sequences, DNA vectors, and DNA delivery protocols [181]. Therefore, it is necessary to expand and diversify the options for heterologous BGC expression, which would increase the probability of success in NP production [178]. As previously described, these tools make use of microbes as models for the expression of recombinant enzymes to increase fructosyltransferase activity. Taken as an example, the isolation and characterization of a levansucrase (M1FT) from *L. mesenteroides* [183]. The M1FT was cloned in the BamHI–EcoRI site of pRSETC to construct pRSET-M1FT. The *E. coli* BL21(DE3) pLysS transformed with pRSET-M1FT was grown to mid-stationary phase at 37 °C. The expressed proteins were purified using Ni–nitrilotriacetic acid (Ni-NTA) agarose. The levansucrase M1FT converted 150 mM sucrose to levan (18%) and FOS, 1-kestose (17%), nystose (11%), and 1,1,1-kestopentaose (7%) [183].

Zymomonas mobilis ZM4 was engineered to produce FOS. For that, two mutant strains were constructed, the *Z. mobilis* ZM4_pB1-L196 and *Z. mobilis* ZM4_pB1-sacB. The first one has a mutated β-fructofuranosidase (Ffase-Leu196) from *Schwanniomyces occidentalis*, and the second one has a native levansucrase (*sac*B). Both strains could produce a mix of FOS with a high concentration of 6-kestose. However, *Z. mobilis* ZM4_pB1-sacB produced 73.4 ± 1.6 g/L of FOS [184].

Another example consists of a fructofuranosidase (Fru6) from *A. arilaitensis* NJEM01 that produces 6-ketose, which was improved by mutagenesis. For this, the catalytic performance of the enzyme Fru6 for the biosynthesis of FOS was predicted by HotSpot Wizard. Thirteen residues were related to the binding site. Double and triple mutants were presented by interactive combinatorial mutagenesis based on the sites S165A, I355A, and H357A. The best screening model was the double mutant S165A/H357A, which, under optimum conditions, could produce 335 g/L of 6-kestose. They suggested that the steric effect of the residue H357 affects the sucrose orientation and its transfructosylation activity [185].

Additionally, an invertase study encoded in the *SUC*2 S2 gene, from *Saccharomyces cerevisiae* S288C [161]. This enzyme was engineered to enhance its fructosyltransferase activity. First, they isolated genomic DNA from *S. cerevisiae*; subsequently, the *SUC*2 gene was amplified from *S. cerevisiae* genomic DNA using the AL530 and AL529 primers. The PCR product was digested with SacI/HindIII and cloned into the pQE80L vector, which provided a tag of His-residues on the N-terminal end of the protein and conferred resistance to ampicillin, resulting in the plasmid SUC2-pQE. *Escherichia coli* XL1-Blue was used as the host strain for standard DNA manipulations. The host strain contained the plasmid pRARE2, which encoded a set of tRNAs corresponding to codons with low frequency in *E. coli*, and it confers resistance to chloramphenicol. Afterward, each plasmid was introduced in *E. coli* Rosetta 2 to optimize the gene expression. Transformants were grown at 37 °C and induced with 5 mM isopropyl-D-1-thiogalactopyranoside (IPTG) for 14 h at 12 °C to achieve the expression of *SUC*2. Subsequently, protein purification was carried out by affinity chromatography. The replacement of amino acids (W19Y, N21S, N24S) in the conserved β-fructofuranosidase site resulted in the increase in 6-kestose production by ten-folds [161]. Moreover, research performed by Marín-Navarro *et al.* (2015) studied an invertase with transfructosylation activity, also from *Saccharomyces cerevisiae*, CECT1624: *MAT*a leu*1* suc^0. The study was focused on the catalytic site of the enzyme. For this, an EcoRI/HindIII fragment containing the *SUC*2 gene from *S. cerevisiae*, was subcloned into YEplac181 to generate the plasmid pSUC-wt. The plasmid with the mutated gene was called pSUC-N21S. The mutant version encodes the N21S amino acid replacement by site-directed mutagenesis. The results showed that the engineered invertase produced 200 g/L of 6-kestose from 60% of sucrose [62].

In this context, filamentous fungi genes with fructosyltransferase activity have been used in yeast to try to overexpress their activity. For instance, Yang *et al.* (2016) evaluated cDNA encoding the FTase gene from *A. niger*, which was inserted to produce the

recombinant protein and heterologously expressed in *P. pastoris*. The *A. niger* FTase cDNA, introns-free, was synthesized by the *SuperScript* III *First-Strand Synthesis System*. The cDNA was used as the template for PCR. The primer sequences contained the EcoRI (50) and NotI (30) restriction sites. The mature FTase gene was obtained as a PCR product, and the vector pPIC9K was digested with EcoRI and NotI and gel-purified. The digested PCR product was then ligated into the pPIC9K. The identity of the recombinant plasmid pPIC9K-fwt was successfully confirmed by restriction analysis and sequencing. The plasmid pPIC9K-fwt was linearized with SacI and then transformed into *P. pastoris* GS115 by electroporation. They reported 1020 U/mL for the activity of recombinant FTase, which was 1160-fold higher than the native FTase from *A. niger* YZ59. In addition, the highest yield of FOS for the recombinant FTase reached 343.3 g/L after 2 h of synthesis [186]. Previously, a FFase from *A. melanogenum* was reported with a β-fructofuranosidase activity of 281.7 $\pm$ 7.1 U/mL. With an overexpression of the gene in yeast, the transformant 33 produces 557.7 U/mL of the β-fructofuranosidase activity. The yield of FOS was 0.66 g of FOS/g of sucrose, and the percentages of GF2, GF3, and GF4 were 79.5%, 18.9%, and 1.6%, respectively [187].

Zhang *et al.* (2022) used the FTase gene (*A. jop*-FTase) on the cell surface of *Y. lipolytica*. First, mannitol and erythritol were synthesized. After that, a remotion of these compounds occurred, and the yeasts were evaluated with a solution of 300 g/L to convert sucrose into FOS at 30 °C on a shaking incubator at 250 rpm. They obtained 85 g/L FOS (GF2, GF3, GF4), with a conversion rate near 60% [188]. Franken et al. (2013) used an invertase, Δ*suc2*, null mutant, and two engineered sucrose accumulating yeast strains as hosts for the expression of the levansucrase (M1FT), previously cloned from the bacteria *Leuconostoc mesenteroides*. Intracellular sucrose accumulation was achieved by the expression of sucrose synthase (Susy) or sucrose transporter (SUT). The results showed that in both Δ*suc2* and sucrose accumulating strains, the levansucrase (M1FT) performed fructosyltransferase activity. In addition, levansucrases (M1FT) have a particular secretion signal sequence in the N-terminal region of the protein. Thus, the result of the deletion of the predicted signal sequence showed a decrease in levan production. Moreover, the co-expression of levansucrase M1FT and SUT produced 7.75 g/L of extracellular levan in a medium with 50 g/L of sucrose, and the TLC analysis showed faint spots below sucrose, which suggested the presence of FOS [189]. Thus, yeast could be a potential model for the heterologous expression of carbohydrates.

These approaches allow the search and detection of novel genes worth exploring for the massive production of FOS. For instance, an *inu*Hj gene from the archae *Halalkalicoccus jeotgali* B3 was amplified and then expressed in *E. coli*. The recombinant FTF produced inulin using sucrose as substrate [130].

This is an opportunity to find microorganisms with outstanding characteristics that could be used as a chassis for the biotechnological production of relevant industrial compounds. These microorganisms could not possess an FTase/FFase enzyme for the transfructosylation of sucrose. Nonetheless, the overexpression of these enzymes by synthetic biology could be an excellent approach to increase the production of FOS by this microorganism. One microorganism that could be used as a system for FOS production is *Saccharomyces cerevisiae.* This yeast has some advantages for the process over other microorganisms. For instance, it requires simple culturing conditions, possesses short replication cycles, and cell recovery is relatively easier than that of filamentous fungi [143]. Thus, the potential massive production of FOS by yeast showed interesting results to be scrutinized [90]. That is, fewer enzymatic steps for fructan production represent a simpler mechanism to mimic or modify. Table 5 shows some of the manipulations to improve the fructosyltransferase activity and their scope.

Table 5. Culturing conditions and modifications for fructan production by yeast.

	Modification			Culturing Conditions			Product			Reference
Modified Microorganism	**Enzyme**	**Method/Technique**	**Activity Increase**	**Culture**	**Component**	**g/L**	**Conditions**	**Name**	**g/L**	**$Y_{P/S}$ (%)**	
Aureobasidium pullulans	FTase	Genetic modification of *A. pullulans* using sequential, random chemical mutagenesis using ethidium bromide and ethyl methane sulfonate.	Increase in 6 and 2-fold extracellular and intracellular FTase, respectively, compared to the wild-type.	Cells	Sucrose Yeast extract $NaNO_3$ KH_2PO_4 NH_4Cl NaCl	200 5 2 1 1 5	Agitation = n.s. pH = 5 T = 45 °C t = 1 h	n.d	n.d.	n.d.	[70]
Aspergillus oryzae S719	FTase	*A. oryaze* S719 irradiation overexpressing a *β*-fructofuranosidase.	n.d.	Enzymatic extract	Sucrose	900	Agitation = 160 pm pH = 6 T = 50 °C t = 20 h	FOS	586	65.11	[152]
Yarrowia lipolytica CGMCC11368	FTase	The *A. oryaze* FTase was displayed on the cell surface of an engineered *Y. lipolytica.*	The yield increased by 10%.	Cells	Sucrose	800	Agitation = n.s. pH = 6 T = 60 °C t = 3 h	FOS kestose neo-kestose nystose fructofuranosylnystose	480	60%	[190]
Pichia pastoris	FTase and *β*-fructanofuranosidase	*T. maritima* FFase and *S. arundinaceus* 1-SST were modified by directed mutagenesis and expressed constitutively in *P. pastoris.*	n.d.	Enzyme	Sucrose Yeast extract	50 0.5	Agitation = 900 rpm pH = 5.5 T = 30 °C t = 72 h	FOS 6-kestose neo-kestose	n.s.	37	[191]

Table 5. *Cont.*

Modification				Culturing Conditions				Product			Reference
Modified Microorganism	**Enzyme**	**Method/Technique**	**Activity Increase**	**Culture**	**Component**	**g/L**	**Conditions**	**Name**	**g/L**	**$Y_{P/S}$ (%)**	
Pichia pastoris GS115	FTase	*A. niger* YZ59 FTase was obtained and expressed in *P. pastoris.*	Increase 1160-fold the activity than the native FTase.	Enzyme	Sucrose	600	Agitation = n.s. pH = 5.5 T = 4z0 °C t = 2 h	FOS	343	57.16	[186]
Pichia pastoris	FTase	*A. terreus* FTase into *K. lactics*, then CRISPR/Cas9 was used to inactive a native INV.	Increase 66.9% the transferase activity.	Cells	FM22 medium: Glucose Galactose Lactose	 30 7 or 7	Agitation = n.s. pH = 6 T = 30 °C t = n.s.	n.d.	n.d.	n.d.	[192]

n.d. = not determined, n.s. = not specified.

5. Conclusions

Fructooligosaccharides possess a worldwide market due to their multiple benefits for human health. On the other hand, the same benefits have greatly increased their demand in the market. Thus, there is a need to find new producers of these prebiotics, which include the use of several types of microorganisms. In addition, these microorganisms must be approached by profitable bioprocesses. To reach such a goal, deeper research in the evaluation and comprehension of exogenous factors, for instance, temperature and nutrients, among others, over the yield and quality of FOS production, are needed. In addition, the insertion of genes codifying for FFases or FTases in more efficient microorganisms at the industrial level must be considered. In this regard, the industrial-scale production of FOS must be increased based on microbial bioreactors due to their operational ease and high yields, which can be further optimized.

6. Future Directions (Perspectives)

Undoubtedly, the maximum optimization of the production of FOS must be directed first to the massive search of microorganisms with fructosyltransferase activity. In addition, the search for more substrate- and product-specific enzymes with higher FOS production rates is contained in such microorganisms. Second, the improvement of protein structures to release the full catalytic potential of FTases and FFases. Third, the search for microorganisms that do not produce FOS but have operative advantages, for instance, fast growth, easy culturing, and their capability to over-express the production of enzymes for FOS synthesis. These would serve as highly efficient chassis containers of enhanced enzymes for FOS production. A fourth stage would consist of the systematic testing of the environmental factors dictating the FOS production kinetics to reach their maximum production. Such a test must be profiled by metabolic fingerprinting coupled with multivariate data analysis and factorial or surface response models. Finally, all the produced knowledge must be integrated and exploited by high-scale bioreactor technology. In this context, archaea represent a whole new world of possibilities for finding new or more efficient enzymes related to fructan synthesis, which could be approached as previously suggested. Furthermore, as mentioned at the beginning of this manuscript, there is a broad structural diversity of fructans. Nonetheless, microorganisms are limited so far to linear $\beta(2\rightarrow1)$ and $\beta(2\rightarrow6)$ structures. In this context, associated bacteria and yeast to agave plants have not been characterized from a chemical perspective to determine if they are also capable of producing branched structures. That is, if associated microorganisms of agaves produce linear fructans, are the highly branched neo-fructans (agavins) exclusively synthesized by plant enzymes? Or can microorganisms synthesize branched fructans when living inside their host plants? Or the origin of agavins is the result of complementary enzymatic systems? That is, microbial FTases and FFases contribute to the linear backbone of agavins, which will be later ramified by other plant enzymes. If so, to what exert agave microbial communities and other bearing branched fructans species contribute to the production and accumulation rate of these molecules? Understanding such correlations might probably serve as a bioinspirational base for the development of new biotechnological approaches for accelerating the production of FOS. In this regard, because of the multifactorial character needed, FOS production improvement, systems biology by means of OMIC approaches, and other high-throughput molecular tools could be a good way to systematically unravel and maximize the production of fructans to fulfill the increasing demand for these carbohydrates as prebiotics.

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References

1. Singh, R.S.; Singh, R.P.; Kennedy, J.F. Recent insights in enzymatic synthesis of fructooligosaccharides from inulin. *Int. J. Biol. Macromol.* **2016**, *85*, 565–572. [CrossRef]
2. Sandoval-González, R.S.; Jiménez-Islas, H.; Navarrete-Bolaños, J.L. Design of a fermentation process for agave fructooligosaccharides production using endo-inulinases produced in situ by *Saccharomyces paradoxus*. *Carbohydr. Polym.* **2018**, *198*, 94–100. [CrossRef]
3. Trujillo, L.E.; Martínez, D.; Pérez, E.; Rivera, L.; Pérez, J.; Pais, J.M. Fructosyltransferases and invertases: Useful enzymes in the food and feed industries. In *Enzymes in Food Biotechnology*; Kuddus, M., Ed.; Academic Press: Cambridge, MA, USA, 2019; pp. 451–469. [CrossRef]
4. Banguela, A.; Hernández, L. Fructans: From natural sources to transgenic plants. *Biotecnol. Apl.* **2006**, *23*, 202–210.
5. Paturi, G.; Butts, C.A.; Bentley-Hewitt, K.L.; Hedderley, D.; Stoklosinski, H.; Ansell, J. Differential effects of probiotics, prebiotics, and synbiotics on gut microbiota and gene expression in rats. *J. Funct. Foods* **2015**, *13*, 204–213. [CrossRef]
6. Wang, M.; Cheong, K.L. Preparation, structural characterization, and bioactivities of fructans: A review. *Molecules* **2023**, *28*, 1613. [CrossRef]
7. Blaut, M. Relationship of prebiotics and food to intestinal microflora. *Eur. J. Nutr.* **2002**, *41*, i11–i16. [CrossRef]
8. Cummings, J.H.; Macfarlane, G.T. The control and consequences of bacterial fermentation in the human colon. *J. Appl. Bacteriol.* **1991**, *70*, 443–459. [CrossRef]
9. Blaak, E.E.; Canfora, E.E.; Theis, S.; Frost, G.; Groen, A.K.; Mithieux, G.; Nauta, A.; Scott, K.; Stahl, B.; Van Harsselaar, J.; et al. Short chain fatty acids in human gut and metabolic health. *Benef. Microbes* **2020**, *11*, 411–455. [CrossRef]
10. Ten Bruggencate, S.J.; Bovee-Oudenhoven, I.M.; Lettink-Wissink, M.L.; Katan, M.B.; van der Meer, R. Dietary fructooligosaccharides affect intestinal barrier function in healthy men. *J. Nutr.* **2006**, *136*, 70–74. [CrossRef]
11. Di Bartolomeo, F.; Van den Ende, W. Fructose and fructans: Opposite effects on health? *Plant Foods Hum. Nutr.* **2015**, *70*, 227–237. [CrossRef]
12. Urías-Silvas, J.E. Efecto Prebiótico de los Fructanos de Agaves y Dasylirion y su Implicación en el Metabolismo de Glucosa y Lípidos en Ratones. Doctoral Thesis, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Irapuato, Mexico, 2008.
13. Scholz-Ahrens, K.E.; Schrezenmeir, J. Inulin and oligofructose and mineral metabolism: The evidence from animal trials. *J. Nutr.* **2007**, *137*, 2513S–2523S. [CrossRef] [PubMed]
14. Vogt, L.; Ramasamy, U.; Meyer, D.; Pullens, G.; Venema, K.; Faas, M.M.; Schols, H.A.; de Vos, P. Immune modulation by different types of $\beta 2 \rightarrow$ 1-fructans is toll-like receptor dependent. *PLoS ONE* **2013**, *8*, e68367. [CrossRef] [PubMed]
15. Espinosa-Andrews, H.; Urías-Silvas, J.E.; Morales-Hernández, N. The role of agave fructans in health and food applications: A review. *Trends Food Sci. Technol.* **2021**, *114*, 585–598. [CrossRef]
16. Gibson, G.R.; Roberfroid, M.B. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J. Nutr.* **1995**, *125*, 1401–1412. [CrossRef]
17. De la Rosa, O.; Flores-Gallegos, A.C.; Muñíz-Marquez, D.; Nobre, C.; Contreras-Esquivel, J.C.; Aguilar, C.N. Fructooligosaccharides production from agro-wastes as alternative low-cost source. *Trends Food Sci. Technol.* **2019**, *91*, 139–146. [CrossRef]
18. Femia, A.P.; Salvadori, M.; Broekaert, W.F.; François, I.E.; Delcour, J.A.; Courtin, C.M.; Caderni, G. Arabinoxylan-oligosaccharides (AXOS) reduce preneoplastic lesions in the colon of rats treated with 1, 2-dimethylhydrazine (DMH). *Eur. J. Nutr.* **2010**, *49*, 127–132. [CrossRef]
19. Xu, B.; Wang, Y.; Li, J.; Lin, Q. Effect of prebiotic xylooligosaccharides on growth performances and digestive enzyme activities of allogynogenetic crucian carp (*Carassius auratus gibelio*). *Fish Physiol. Biochem.* **2009**, *35*, 351–357. [CrossRef]
20. Kruschitz, A.; Nidetzky, B. Downstream processing technologies in the biocatalytic production of oligosaccharides. *Biotechnol. Adv.* **2020**, *43*, 107568. [CrossRef]
21. Nguyen, T.T. The cholesterol-lowering action of plant stanol esters. *J. Nutr.* **1999**, *129*, 2109–2112. [CrossRef]
22. Wang, Y.; Zeng, T.; Wang, S.E.; Wang, W.; Wang, Q.; Yu, H.X. Fructo-oligosaccharides enhance the mineral absorption and counteract the adverse effects of phytic acid in mice. *Nutrition* **2010**, *26*, 305–311. [CrossRef]

23. Costa, G.T.; Vasconcelos, Q.D.; Aragão, G.F. Fructooligosaccharides on inflammation, immunomodulation, oxidative stress, and gut immune response: A systematic review. *Nutr. Rev.* **2022**, *80*, 709–722. [CrossRef] [PubMed]
24. Guerra, L.; Ureta, M.; Romanini, D.; Woitovich, N.; Gómez-Zavaglia, A.; Clementz, A. Enzymatic synthesis of fructooligosaccharides: From carrot discards to prebiotic juice. *Food Res. Int.* **2023**, *170*, 112991. [CrossRef] [PubMed]
25. Transparencymarketresearch.com. Prebiotics Ingredients Market: Health and Wellness Spending Continues to Create Opportunities. Available online: http://www.transparencymarketresearch.com/prebiotics-market.html (accessed on 13 October 2023).
26. Grandviewresearch.com. Prebiotics Market Size, Share & Trends Analysis Report by Ingredients (FOS, Inulin, GOS, MOS), By Application (Food & Beverages, Dietary Supplements, Animal Feed), By Region, and Segment Forecasts, 2022–2030. Available online: https://www.grandviewresearch.com/industry-analysis/prebiotics-market (accessed on 13 October 2023).
27. Reportlinker.com. Prebiotics Market Analysis by Ingredient (FOS, inulin, GOS, MOS), By Application (Food and Beverages, Animal Feed, Dietary Supplements) and Segmenta Forescasts to 2024. Available online: https://www.reportlinker.com/p03937911/Prebiotics-Market-Analysis-By-Ingredient-FOS-Inulin-GOS-MOS-By-Application-Food-Beverages-Animal-Feed-Dietary-Supplements-And-Segment-Forecasts-To.html (accessed on 13 October 2023).
28. Verifiedmarketresearch.com. Fructooligosaccharide (FOS) Market Size and Forecast. Available online: https://www.verifiedmarketresearch.com/product/global-fructooligosaccharide-fos-market-size-and-forecast/#:~:text=Fructooligosaccharide%20(FOS)%20Market%20size%20was,10.5%25%20from%202023%20to%202030 (accessed on 13 October 2023).
29. Van Laere, A.; Van Den Ende, W. Inulin metabolism in dicots: Chicory as a model system. *Plant Cell Environ.* **2002**, *25*, 803–813. [CrossRef]
30. Ende, W.V.D. Multifunctional fructans and raffinose family oligosaccharides. *Front. Plant Sci.* **2013**, *4*, 247. [CrossRef]
31. McCallum, J.; Clarke, A.; Pither-Joyce, M.; Shaw, M.; Butler, R.; Brash, D.; Scheffer, J.; Sims, I.; van Heusden, S.; Shigyo, M.; et al. Genetic mapping of a major gene affecting onion bulb fructan content. *Theor. Appl. Genet.* **2006**, *112*, 958–967. [CrossRef]
32. Shiomi, N.; Onodera, S.; Chatterton, N.J.; Harrison, P.A. Separation of fructooligosaccharide isomers by anion-exchange chromatography. *Agric. Biol. Chem.* **1991**, *55*, 1427–1428. [CrossRef]
33. Viera-Alcaide, I.; Hamdi, A.; Guillén-Bejarano, R.; Rodríguez-Arcos, R.; Espejo-Calvo, J.A.; Jiménez-Araujo, A. Asparagus roots: From an agricultural by-product to a valuable source of fructans. *Foods* **2022**, *11*, 652. [CrossRef]
34. Sims, I.M. Structural diversity of fructans from members of the order Asparagales in New Zealand. *Phytochemistry* **2003**, *63*, 351–359. [CrossRef]
35. Cimini, S.; Locato, V.; Vergauwen, R.; Paradiso, A.; Cecchini, C.; Vandenpoel, L.; Verspreet, J.; Courtin, C.M.; D'Egidio, M.G.; Ende, W.V.D.; et al. Fructan biosynthesis and degradation as part of plant metabolism controlling sugar fluxes during durum wheat kernel maturation. *Front. Plant Sci.* **2015**, *6*, 89. [CrossRef]
36. Abeynayake, S.W.; Etzerodt, T.P.; Jonavičienė, K.; Byrne, S.; Asp, T.; Boelt, B. Fructan metabolism and changes in fructan composition during cold acclimation in perennial ryegrass. *Front. Plant Sci.* **2015**, *6*, 329. [CrossRef]
37. López, M.G.; Mancilla-Margalli, N.A.; Mendoza-Díaz, G. Molecular structures of fructans from *Agave tequilana* Weber var. azul. *J. Agric. Food Chem.* **2003**, *51*, 7835–7840. [CrossRef] [PubMed]
38. Mancilla-Margalli, N.A.; López, M.G. Water-soluble carbohydrates and fructan structure patterns from Agave and Dasylirion species. *J. Agric. Food Chem.* **2006**, *54*, 7832–7839. [CrossRef] [PubMed]
39. Huazano-García, A.; Silva-Adame, M.B.; Vázquez-Martínez, J.; Gastelum-Arellanez, A.; Sánchez-Segura, L.; López, M.G. Highly branched neo-fructans (Agavins) attenuate metabolic endotoxemia and low-grade inflammation in association with gut microbiota modulation on high-fat diet-fed mice. *Foods* **2020**, *9*, 1792. [CrossRef] [PubMed]
40. Santiago-García, P.A.; López, M.G. Agavins from *Agave angustifolia* and *Agave potatorum* affect food intake, body weight gain and satiety-related hormones (GLP-1 and ghrelin) in mice. *Food Funct.* **2014**, *5*, 3311–3319. [CrossRef] [PubMed]
41. Li, J.; Wang, Y.; Zhang, X.; Cao, L.; Ji, J.; Zheng, Q.; Gao, J. Isolation and structural identification of a novel fructan from *Radix Codonopsis*. *J. Carbohydr. Chem.* **2020**, *39*, 163–174. [CrossRef]
42. Ávila-Fernández, Á.; Olvera-Carranza, C.; Rudino-Pinera, E.; Cassab, G.I.; Nieto-Sotelo, J.; López-Munguía, A. Molecular characterization of sucrose: Sucrose 1-fructosyltransferase (1-SST) from *Agave tequilana* Weber var. azul. *Plant Sci.* **2007**, *173*, 478–486. [CrossRef]
43. Tezgel, N.; Kırtel, O.; Van den Ende, W.; Toksoy Öner, E. Fructosyltransferase enzymes for microbial fructan production. In *Microbial Enzymes: Roles and Applications in Industries*; Arora, N., Mischra, J., Mischra, V., Eds.; Springer: Singapore, 2020; Volume 11, pp. 1–39. [CrossRef]
44. Márquez-López, R.E.; Loyola-Vargas, V.M.; Santiago-García, P.A. Interaction between fructan metabolism and plant growth regulators. *Planta* **2022**, *255*, 49. [CrossRef]
45. Sangeetha, P.T.; Ramesh, M.N.; Prapulla, S.G. Recent trends in the microbial production, analysis, and application of fructooligosaccharides. *Trends Food Sci. Technol.* **2005**, *16*, 442–457. [CrossRef]
46. Álvaro-Benito, M.; de Abreu, M.; Fernández-Arrojo, L.; Plou, F.J.; Jiménez-Barbero, J.; Ballesteros, A.; Polaina, J.; Fernández-Lobato, M. Characterization of a β-fructofuranosidase from *Schwanniomyces occidentalis* with transfructosylating activity yielding the prebiotic 6-kestose. *J. Biotechnol.* **2007**, *132*, 75–81. [CrossRef]
47. Choukade, R.; Kango, N. Production, properties, and applications of fructosyltransferase: A current appraisal. *Crit. Rev. Biotechnol.* **2021**, *41*, 1178–1193. [CrossRef]

48. Nobre, C.; Teixeira, J.A.; Rodrigues, L.R. New trends and technological challenges in the industrial production and purification of fructo-oligosaccharides. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 1444–1455. [CrossRef] [PubMed]
49. Sánchez-Martínez, M.J.; Soto-Jover, S.; Antolinos, V.; Martínez-Hernández, G.B.; López-Gómez, A. Manufacturing of short-chain fructooligosaccharides: From laboratory to industrial scale. *Food Eng. Rev.* **2020**, *12*, 149–172. [CrossRef]
50. Eskandari Nasab, E.; Habibi-Rezaei, M.; Khaki, A.; Balvardi, M. Investigation on acid hydrolysis of inulin: A response surface methodology approach. *Int. J. Food Eng.* **2009**, *5*, 1–8. [CrossRef]
51. Sarchami, T.; Rehmann, L. Optimizing acid hydrolysis of Jerusalem artichoke-derived inulin for fermentative butanol production. *BioEnergy Res.* **2015**, *8*, 1148–1157. [CrossRef]
52. Ricca, E.; Calabrò, V.; Curcio, S.; Iorio, G. The state of the art in the production of fructose from inulin enzymatic hydrolysis. *Crit. Rev. Biotechnol.* **2007**, *27*, 129–145. [CrossRef]
53. Kochhar, A.; Gupta, A.K.; Kaur, N. Purification and immobilisation of inulinase from *Aspergillus candidus* for producing fructose. *J. Sci. Food Agric.* **1999**, *79*, 549–554. [CrossRef]
54. Cheng, M.; Wu, H.; Zhang, W.; Mu, W. Difructose anhydride III: A 50-year perspective on its production and physiological functions. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 6714–6725. [CrossRef]
55. Canatar, M.; Tufan, H.N.G.; Ünsal, S.B.E.; Koc, C.Y.; Ozcan, A.; Kucuk, G.; Basmak, S.; Yatmaz, E.; Germec, M.; Yavuz, I.; et al. Inulinase and fructooligosaccharide production from carob using *Aspergillus niger* A42 (ATCC 204447) under solid-state fermentation conditions. *Int. J. Biol. Macromol.* **2023**, *245*, 125520. [CrossRef]
56. Schorsch, J.; Castro, C.C.; Couto, L.D.; Nobre, C.; Kinnaert, M. Optimal control for fermentative production of fructo-oligosaccharides in fed-batch bioreactor. *J. Process Control.* **2019**, *78*, 124–138. [CrossRef]
57. Ganaie, M.A.; Lateef, A.; Gupta, U.S. Enzymatic trends of fructooligosaccharides production by microorganisms. *Appl. Biochem. Biotechnol.* **2014**, *172*, 2143–2159. [CrossRef]
58. Kushi, R.T.; Monti, R.; Contiero, J. Production, purification, and characterization of an extracellular inulinase from *Kluyveromyces marxianus* var. bulgaricus. *J. Ind. Microbiol. Biotechnol.* **2000**, *25*, 63–69. [CrossRef]
59. Karboune, S.; Appanah, N.; Khodaei, N.; Tian, F. Enzymatic synthesis of fructooligosaccharides from sucrose by endo-inulinase-catalyzed transfructosylation reaction in biphasic systems. *Process Biochem.* **2018**, *69*, 82–91. [CrossRef]
60. Maiorano, A.E.; Piccoli, R.M.; Da Silva, E.S.; de Andrade Rodrigues, M.F. Microbial production of fructosyltransferases for synthesis of prebiotics. *Biotechnol. Lett.* **2008**, *30*, 1867–1877. [CrossRef] [PubMed]
61. Ghazi, I.; Fernandez-Arrojo, L.; Garcia-Arellano, H.; Ferrer, M.; Ballesteros, A.; Plou, F.J. Purification and kinetic characterization of a fructosyltransferase from *Aspergillus aculeatus*. *J. Biotechnol.* **2007**, *128*, 204–211. [CrossRef]
62. Marín-Navarro, J.; Talens-Perales, D.; Polaina, J. One-pot production of fructooligosaccharides by a *Saccharomyces cerevisiae* strain expressing an engineered invertase. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 2549–2555. [CrossRef]
63. Magri, A.; Oliveira, M.R.; Baldo, C.; Tischer, C.A.; Sartori, D.; Mantovani, M.S.; Celligoi, M.A.P.C. Production of fructooligosaccharides by *Bacillus subtilis* natto CCT7712 and their antiproliferative potential. *J. Appl. Microbiol.* **2020**, *128*, 1414–1426. [CrossRef]
64. Vicente-Magueyal, F.J.; Bautista-Méndez, A.; Villanueva-Tierrablanca, H.D.; García-Ruíz, J.L.; Jiménez-Islas, H.; Navarrete-Bolaños, J.L. Novel process to obtain agave sap (aguamiel) by directed enzymatic hydrolysis of agave juice fructans. *LWT* **2020**, *127*, 109387. [CrossRef]
65. Picazo, B.; Flores-Gallegos, A.C.; Muñiz-Márquez, D.B.; Flores-Maltos, A.; Michel-Michel, M.R.; de la Rosa, O.; Rodriguez-Jasso, R.M.A.I.; Rodriguez, R.; Aguilar-González, C.N. Enzymes for fructooligosaccharides production: Achievements and opportunities. In *Enzymes in Food Biotechnology*; Kuddus, M., Ed.; Academic Press: Cambridge, MA, USA, 2019; pp. 303–320. [CrossRef]
66. Schultz, G.; Alexander, R.; Lima, F.V.; Giordano, R.C.; Ribeiro, M.P. Kinetic modeling of the enzymatic synthesis of galacto-oligosaccharides: Describing galactobiose formation. *Food Bioprod. Process.* **2021**, *127*, 1–13. [CrossRef]
67. Van Hijum, S.A.F.T.; van Geel-Schutten, G.H.; Rahaoui, H.; Van der Maarel, M.J.E.C.; Dijkhuizen, L. Characterization of a novel fructosyltransferase from *Lactobacillus reuteri* that synthesizes high-molecular-weight inulin and inulin oligosaccharides. *Appl. Environ. Microbiol.* **2002**, *68*, 4390–4398. [CrossRef]
68. Antošová, M.; Polakovič, M. Fructosyltransferases: The enzymes catalyzing production of fructooligosaccharides. *Chem. Pap* **2001**, *55*, 350–358.
69. Alméciga-Díaz, C.J.; Gutiérrez, Á.M.; Bahamon, I.; Rodríguez, A.; Rodríguez, M.A.; Sánchez, O.F. Computational analysis of the fructosyltransferase enzymes in plants, fungi, and bacteria. *Gene* **2011**, *484*, 26–34. [CrossRef] [PubMed]
70. Ademakinwa, A.N.; Ayinla, Z.A.; Agboola, F.K. Strain improvement and statistical optimization as a combined strategy for improving fructosyltransferase production by *Aureobasidium pullulans* NAC8. *J. Genet. Eng. Biotechnol.* **2017**, *15*, 345–358. [CrossRef] [PubMed]
71. L'Hocine, L.; Wang, Z.; Jiang, B.; Xu, S. Purification and partial characterization of fructosyltransferase and invertase from *Aspergillus niger* AS0023. *J. Biotechnol.* **2000**, *81*, 73–84. [CrossRef]
72. Kırtel, O.; Lescrinier, E.; Van den Ende, W.; Öner, E.T. Discovery of fructans in Archaea. *Carbohydr. Polym.* **2019**, *220*, 149–156. [CrossRef] [PubMed]
73. Versluys, M.; Kirtel, O.; Toksoy Öner, E.; Van den Ende, W. The fructan syndrome: Evolutionary aspects and common themes among plants and microbes. *Plant Cell Environ.* **2018**, *41*, 16–38. [CrossRef] [PubMed]

74. Ko, H.; Sung, B.H.; Kim, M.J.; Sohn, J.H.; Bae, J.H. Fructan biosynthesis by yeast cell factories. *J. Microbiol. Biotechnol.* **2022**, *32*, 1373. [CrossRef] [PubMed]
75. Vijn, I.; Smeekens, S. Fructan: More than a reserve carbohydrate? *Plant Physiology.* **1999**, *120*, 351–360. [CrossRef] [PubMed]
76. Lammens, W.; Le Roy, K.; Schroeven, L.; Van Laere, A.; Rabijns, A.; Van den Ende, W. Structural insights into glycoside hydrolase family 32 and 68 enzymes: Functional implications. *J. Exp. Bot.* **2009**, *60*, 727–740. [CrossRef]
77. Anwar, M.A.; Kralj, S.; van der Maarel, M.J.; Dijkhuizen, L. The probiotic *Lactobacillus johnsonii* NCC 533 produces high-molecular-mass inulin from sucrose by using an inulosucrase enzyme. *Appl. Environ. Microbiol.* **2008**, *74*, 3426–3433. [CrossRef]
78. Olivares-Illana, V.; Wacher-Rodarte, C.; Le Borgne, S.; López-Munguía, A. Characterization of a cell-associated inulosucrase from a novel source: A *Leuconostoc citreum* strain isolated from Pozol, a fermented corn beverage of Mayan origin. *J. Ind. Microbiol. Biotechnol.* **2002**, *28*, 112–117. [CrossRef]
79. Ponstein, A.S.; Van Leeuwen, M.B. *In vitro* synthesis of inulin by the inulosucrase from *Streptococcus mutans*. *Stud. Plant Sci.* **1993**, *3*, 281–287. [CrossRef]
80. Malik, A. Molecular cloning and in silico characterization of fructansucrase gene from *Weissella confusa* MBFCNC-2(1) isolated from local beverage. *Asia-Pac. J. Mol. Biol. Biotechnol.* **2012**, *20*, 33–42.
81. Támbara, Y.; Hormaza, J.V.; Pérez, C.; León, A.; Arrieta, J.; Hernández, L. Structural analysis and optimized production of fructo-oligosaccharides by levansucrase from *Acetobacter diazotrophicus* SRT4. *Biotechnol. Lett.* **1999**, *21*, 117–121. [CrossRef]
82. Chambert, R.; Treboul, G.; Dedonder, R. Kinetic studies of levansucrase of *Bacillus subtilis*. *Eur. J. Biochem.* **1974**, *41*, 285–300. [CrossRef] [PubMed]
83. Inthanavong, L.; Tian, F.; Khodadadi, M.; Karboune, S. Properties of *Geobacillus stearothermophilus* levansucrase as potential biocatalyst for the synthesis of levan and fructooligosaccharides. *Biotechnol. Prog.* **2013**, *29*, 1405–1415. [CrossRef]
84. Van Hijum, S.A.F.T.; Szalowska, E.; Van Der Maarel, M.J.E.C.; Dijkhuizen, L. Biochemical and molecular characterization of a levansucrase from *Lactobacillus reuteri*. *Microbiology* **2004**, *150*, 621–630. [CrossRef] [PubMed]
85. Morales-Arrieta, S.; Rodríguez, M.E.; Segovia, L.; López-Munguía, A.; Olvera-Carranza, C. Identification and functional characterization of levS, a gene encoding for a levansucrase from *Leuconostoc mesenteroides* NRRL B-512 F. *Gene* **2006**, *376*, 59. [CrossRef]
86. Hettwer, U.; Gross, M.; Rudolph, K. Purification and characterization of an extracellular levansucrase from *Pseudomonas syringae* pv. phaseolicola. *J. Bacteriol.* **1995**, *177*, 2834–2839. [CrossRef]
87. Jung, H.C.; Lebeault, J.M.; Pan, J.G. Surface display of *Zymomonas mobilis* levansucrase by using the ice-nucleation protein of *Pseudomonas syringae*. *Nat. Biotechnol.* **1998**, *16*, 576–580. [CrossRef]
88. Yoshikawa, J.; Amachi, S.; Shinoyama, H.; Fujii, T. Multiple β-fructofuranosidases by *Aureobasidium pullulans* DSM2404 and their roles in fructooligosaccharide production. *FEMS Microbiol. Lett.* **2006**, *265*, 159–163. [CrossRef]
89. Zeng, W.; Leng, S.; Zhang, Y.; Chen, G.; Liang, Z. Development of new strategies for the production of high-purity fructooligosaccharides using β-fructofuranosidase and a novel isolated *Wickerhamomyces anomalus*. *Sep. Purif. Technol.* **2022**, *284*, 120236. [CrossRef]
90. Yoshikawa, J.; Honda, Y.; Saito, Y.; Sato, D.; Iwata, K.; Amachi, S.; Kashiwagi, Y.; Maehashi, K. Isolation and identification of Zalaria sp. Him3 as a novel fructooligosaccharides-producing yeast. *J. Appl. Microbiol.* **2022**, *132*, 1104–1111. [CrossRef] [PubMed]
91. Gimeno-Pérez, M.; Linde, D.; Fernández-Arrojo, L.; Plou, F.J.; Fernández-Lobato, M. Heterologous overproduction of β-fructofuranosidase from yeast *Xanthophyllomyces dendrorhous*, an enzyme producing prebiotic sugars. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 3459–3467. [CrossRef] [PubMed]
92. Patel, V.; Saunders, G.; Bucke, C. N-Deglycosylation of fructosyltransferase and invertase from *Fusarium oxysporum* decreases stability but has little effect on kinetics and synthetic specificity. *Biotechnol. Lett.* **1997**, *19*, 75–77. [CrossRef]
93. Dhake, A.B.; Patil, M.B. Effect of substrate feeding on production of fructosyltransferase by *Penicillium purpurogenum*. *Braz. J. Microbiol.* **2007**, *38*, 194–199. [CrossRef]
94. Lateef, A.; Gueguim-Kana, E.B. Utilization of cassava wastes in the production of fructosyltransferase by *Rhizopus stolonifer* LAU 07. *Rom. Biotechnol. Lett.* **2012**, *17*, 7309–7316.
95. Xie, Y.; Zhou, H.; Liu, C.; Zhang, J.; Li, N.; Zhao, Z.; Sun, G.; Zhong, Y. A molasses habitat-derived fungus *Aspergillus tubingensis* XG21 with high β-fructofuranosidase activity and its potential use for fructooligosaccharides production. *AMB Express* **2017**, *7*, 128. [CrossRef]
96. Nascimento, A.K.C.; Nobre, C.; Cavalcanti, M.T.H.; Teixeira, J.A.; Porto, A.L.F. Screening of fungi from the genus Penicillium for production of β-fructofuranosidase and enzymatic synthesis of fructooligosaccharides. *J. Mol. Catal. B Enzym.* **2016**, *134*, 70–78. [CrossRef]
97. Gimeno-Pérez, M.; Merdzo, Z.; Castillo-Rosa, E.; Hijas, C.M.D.; Fernández-Lobato, M. The β-fructofuranosidase from *Rhodotorula dairenensis*: Molecular cloning, heterologous expression, and evaluation of its transferase activity. *Catalysts* **2021**, *11*, 476. [CrossRef]
98. López, M.G.; Salomé-Abarca, L.F. The agavins (agave carbohydrate) story. *Carbohydr. Polym.* **2023**, *in press*.
99. Xu, W.; Ni, D.; Zhang, W.; Guang, C.; Zhang, T.; Mu, W. Recent advances in levansucrase and inulosucrase: Evolution, characteristics, and application. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 3630–3647. [CrossRef]
100. Porras-Domínguez, J.R.; Ávila-Fernández, Á.; Rodríguez-Alegría, M.E.; Miranda-Molina, A.; Escalante, A.; González-Cervantes, R.; Olvera, C.; Munguía, A.L. Levan-type FOS production using a *Bacillus licheniformis* endolevanase. *Process Biochem.* **2014**, *49*, 783–790. [CrossRef]

101. Santos-Moriano, P.; Fernandez-Arrojo, L.; Poveda, A.; Jimenez-Barbero, J.; Ballesteros, A.O.; Plou, F.J. Levan versus fructooligosaccharide synthesis using the levansucrase from *Zymomonas mobilis*: Effect of reaction conditions. *J. Mol. Catal. B Enzym.* **2015**, *119*, 18–25. [CrossRef]
102. De Siqueira, E.C.; Öner, E.T. Co-production of levan with other high-value bioproducts: A review. *Int. J. Biol. Macromol.* **2023**, *235*, 123800. [CrossRef] [PubMed]
103. Nguyen, H.T.; Nguyen, T.H.; Otto, M. The staphylococcal exopolysaccharide PIA–Biosynthesis and role in biofilm formation, colonization, and infection. *Comput. Struct. Biotechnol. J.* **2020**, *18*, 3324–3334. [CrossRef] [PubMed]
104. de Oliveira, M.R.; da Silva, R.S.S.F.; Buzato, J.B.; Celligoi, M.A.P.C. Study of levan production by *Zymomonas mobilis* using regional low-cost carbohydrate sources. *Biochem. Eng. J.* **2007**, *37*, 177–183. [CrossRef]
105. Ritsema, T.; Smeekens, S. Fructans: Beneficial for plants and humans. *Curr. Opin. Plant Biol.* **2003**, *6*, 223–230. [CrossRef]
106. Visnapuu, T.; Mardo, K.; Alamäe, T. Levansucrases of a *Pseudomonas syringae* pathovar as catalysts for the synthesis of potentially prebiotic oligo-and polysaccharides. *New Biotechnol.* **2015**, *32*, 597–605. [CrossRef]
107. Li, W.; Yu, S.; Zhang, T.; Jiang, B.; Mu, W. Recent novel applications of levansucrases. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 6959–6969. [CrossRef]
108. Öner, E.T.; Hernández, L.; Combie, J. Review of levan polysaccharide: From a century of past experiences to future prospects. *Biotechnol. Adv.* **2016**, *34*, 827–844. [CrossRef]
109. Shih, L.; Chen, L.D.; Wu, J.Y. Levan production using *Bacillus subtilis* natto cells immobilized on alginate. *Carbohydr. Polym.* **2010**, *82*, 111–117. [CrossRef]
110. Xavier, J.R.; Ramana, K.V. Optimization of levan production by cold-active *Bacillus licheniformis* ANT 179 and fructooligosaccharide synthesis by its levansucrase. *Appl. Biochem. Biotechnol.* **2017**, *181*, 986–1006. [CrossRef] [PubMed]
111. Wahyuningrum, D.; Hertadi, R. Isolation and characterization of levan from moderate halophilic bacteria *Bacillus licheniformis* BK AG21. *Procedia Chem.* **2015**, *16*, 292–298. [CrossRef]
112. Djurić, A.; Gojgić-Cvijović, G.; Jakovljević, D.; Kekez, B.; Kojić, J.S.; Mattinen, M.L.; Harju, I.E.; Vrvić, M.M.; Beškoski, V.P. Brachybacterium sp. CH-KOV3 isolated from an oil-polluted environment–a new producer of levan. *Int. J. Biol. Macromol.* **2017**, *104*, 311–321. [CrossRef] [PubMed]
113. Erkorkmaz, B.A.; Kırtel, O.; Duru, Ö.A.; Öner, E.T. Development of a cost-effective production process for Halomonas levan. *Bioprocess Biosyst. Eng.* **2018**, *41*, 1247–1259. [CrossRef]
114. van Hijum, S.A.; Bonting, K.; van der Maarel, M.J.; Dijkhuizen, L. Purification of a novel fructosyltransferase from *Lactobacillus reuteri* strain 121 and characterization of the levan produced. *FEMS Microbiol. Lett.* **2001**, *205*, 323–328. [CrossRef]
115. Liu, J.; Luo, J.; Ye, H.; Zeng, X. Preparation, antioxidant, and antitumor activities in vitro of different derivatives of levan from endophytic bacterium *Paenibacillus polymyxa* EJS-3. *Food Chem. Toxicol.* **2012**, *50*, 767–772. [CrossRef]
116. Simms, P.J.; Boyko, W.J.; Edwards, J.R. The structural analysis of a levan produced by *Streptococcus salivarius* SS2. *Carbohydr. Res.* **1990**, *208*, 193–198. [CrossRef]
117. Nasir, A.; Sattar, F.; Ashfaq, I.; Lindemann, S.R.; Chen, M.H.; Van den Ende, W.; Öner, E.T.; Kirtel, O.; Khaliq, S.; Ghauri, M.A.; et al. Production and characterization of a high molecular weight levan and fructooligosaccharides from a rhizospheric isolate of *Bacillus aryabhattai*. *LWT* **2020**, *123*, 109093. [CrossRef]
118. Gojgic-Cvijovic, G.D.; Jakovljevic, D.M.; Loncarevic, B.D.; Todorovic, N.M.; Pergal, M.V.; Ciric, J.; Loos, K.; Beskoski, V.; Vrvic, M.M. Production of levan by *Bacillus licheniformis* NS032 in sugar beet molasses-based medium. *Int. J. Biol. Macromol.* **2019**, *121*, 142–151. [CrossRef]
119. Xu, W.; Liu, Q.; Bai, Y.; Yu, S.; Zhang, T.; Jiang, B.; Mu, W. Physicochemical properties of a high molecular weight levan from Brenneria sp. EniD312. *Int. J. Biol. Macromol.* **2018**, *109*, 810–818. [CrossRef] [PubMed]
120. Kralj, S.; Leeflang, C.; Sierra, E.I.; Kempiński, B.; Alkan, V.; Kolkman, M. Synthesis of fructooligosaccharides (FosA) and inulin (InuO) by GH68 fructosyltransferases from *Bacillus agaradhaerens* strain WDG185. *Carbohydr. Polym.* **2018**, *179*, 350–359. [CrossRef] [PubMed]
121. Aduse-Opuko, J.; Gilpin, M.L.; Russell, R.R.B. Genetic and antigenic comparison of *Streptococcus mutans* fructosyltransferase and glucan-binding protein. *FEMS Microbiol. Lett.* **1989**, *59*, 279–282. [CrossRef]
122. Rosell, K.G.; Birkhed, D. An inulin-like fructan produced by *Streptococcus mutans*, strain JC2. *Acta Chem. Scand. B* **1974**, *28*, 589. [CrossRef]
123. Loesche, W.J. The identification of bacteria associated with periodontal disease and dental caries by enzymatic methods. *Oral Microbiol. Immunol.* **1986**, *1*, 65–70. [CrossRef]
124. Ni, D.; Xu, W.; Zhu, Y.; Zhang, W.; Zhang, T.; Guang, C.; Mu, W. Inulin and its enzymatic production by inulosucrase: Characteristics, structural features, molecular modifications and applications. *Biotechnol. Adv.* **2019**, *37*, 306–318. [CrossRef]
125. Olivares-Illana, V.; López-Munguía, A.; Olvera, C. Molecular characterization of inulosucrase from *Leuconostoc citreum*: A fructosyltransferase within a glucosyltransferase. *J. Bacteriol.* **2003**, *185*, 3606–3612. [CrossRef]
126. Bersaneti, G.T.; Pan, N.C.; Baldo, C.; Celligoi, M.A.P.C. Co-production of fructooligosaccharides and levan by levansucrase from *Bacillus subtilis* natto with potential application in the food industry. *Appl. Biochem. Biotechnol.* **2018**, *184*, 838–851. [CrossRef]
127. Zhang, T.; Li, R.; Qian, H.; Mu, W.; Miao, M.; Jiang, B. Biosynthesis of levan by levansucrase from *Bacillus methylotrophicus* SK 21.002. *Carbohydr. Polym.* **2014**, *101*, 975–981. [CrossRef]

128. Liu, J.; Luo, J.; Ye, H.; Sun, Y.; Lu, Z.; Zeng, X. Medium optimization and structural characterization of exopolysaccharides from endophytic bacterium *Paenibacillus polymyxa* EJS-3. *Carbohydr. Polym.* **2010**, *79*, 206–213. [CrossRef]
129. Silbir, S.; Dagbagli, S.; Yegin, S.; Baysal, T.; Goksungur, Y. Levan production by *Zymomonas mobilis* in batch and continuous fermentation systems. *Carbohydr. Polym.* **2014**, *99*, 454–461. [CrossRef] [PubMed]
130. Ghauri, K.; Ali, H.; Munawar, N.; Ghauri, M.A.; Anwar, M.A. Glycoside hydrolase family 68 gene of halophilic archaeon *Halalkalicoccus jeotgali* B3T codes for an inulosucrase enzyme. *Biocatal. Biotransformation* **2021**, *39*, 206–213. [CrossRef]
131. Kırtel, O.; Versluys, M.; Van den Ende, W.; Öner, E.T. Fructans of the saline world. *Biotechnol. Adv.* **2018**, *36*, 1524–1539. [CrossRef]
132. Hirabayashi, K.; Kondo, N.; Toyota, H.; Hayashi, S. Production of the functional trisaccharide 1-kestose from cane sugar molasses using *Aspergillus japonicus* β-fructofuranosidase. *Curr. Microbiol.* **2017**, *74*, 145–148. [CrossRef]
133. de Almeida, M.N.; Guimarães, V.M.; Falkoski, D.L.; de Camargo, B.R.; Fontes-Sant'ana, G.C.; Maitan-Alfenas, G.P.; de Rezende, S.T. Purification and characterization of an invertase and a transfructosylase from *Aspergillus terreus*. *J. Food Biochem.* **2018**, *42*, e12551. [CrossRef]
134. Ganaie, M.A.; Rawat, H.K.; Wani, O.A.; Gupta, U.S.; Kango, N. Immobilization of fructosyltransferase by chitosan and alginate for efficient production of fructooligosaccharides. *Process Biochem.* **2014**, *49*, 840–844. [CrossRef]
135. Sánchez, O.; Guio, F.; Garcia, D.; Silva, E.; Caicedo, L. Fructooligosaccharides production by Aspergillus sp. N74 in a mechanically agitated airlift reactor. *Food Bioprod. Process.* **2008**, *86*, 109–115. [CrossRef]
136. Mostafa, F.A.; Wahab, W.A.A.; Salah, H.A.; Nawwar, G.A.; Esawy, M.A. Kinetic and thermodynamic characteristic of *Aspergillus awamori* EM66 levansucrase. *Int. J. Biol. Macromol.* **2018**, *119*, 232–239. [CrossRef]
137. Muñiz-Márquez, D.B.; Contreras, J.C.; Rodríguez, R.; Mussatto, S.I.; Teixeira, J.A.; Aguilar, C.N. Enhancement of fructosyltransferase and fructooligosaccharides production by *A. oryzae* DIA-MF in Solid-State Fermentation using aguamiel as culture medium. *Bioresour. Technol.* **2016**, *213*, 276–282. [CrossRef]
138. Antošová, M.; Illeová, V.; Vandáková, M.; Družkovská, A.; Polakovič, M. Chromatographic separation and kinetic properties of fructosyltransferase from *Aureobasidium pullulans*. *J. Biotechnol.* **2008**, *135*, 58–63. [CrossRef]
139. Ghazi, I.; De Segura, A.G.; Fernández-Arrojo, L.; Alcalde, M.; Yates, M.; Rojas-Cervantes, M.L.; Plou, F.J.; Ballesteros, A. Immobilisation of fructosyltransferase from *Aspergillus aculeatus* on epoxy-activated Sepabeads EC for the synthesis of fructo-oligosaccharides. *J. Mol. Catal. B Enzym.* **2005**, *35*, 19–27. [CrossRef]
140. Ojwach, J.; Kumar, A.; Mukaratirwa, S.; Mutanda, T. Fructooligosaccharides synthesized by fructosyltransferase from an indigenous coprophilous *Aspergillus niger* strain XOBP48 exhibits antioxidant activity. *Bioact. Carbohydr. Diet. Fibre* **2020**, *24*, 100238. [CrossRef]
141. Van der Klei, I.J.; Veenhuis, M. Yeast and filamentous fungi as model organisms in microbody research. *Biochim. Et Biophys. Acta (BBA)-Mol. Cell Res.* **2006**, *1763*, 1364–1373. [CrossRef] [PubMed]
142. Smedsgaard, J.; Nielsen, J. Metabolite profiling of fungi and yeast: From phenotype to metabolome by MS and informatics. *J. Exp. Bot.* **2005**, *56*, 273–286. [CrossRef]
143. Khatun, M.S.; Hassanpour, M.; Mussatto, S.I.; Harrison, M.D.; Speight, R.E.; O'Hara, I.M.; Zhang, Z. Transformation of sugarcane molasses into fructooligosaccharides with enhanced prebiotic activity using whole-cell biocatalysts from *Aureobasidium pullulans* FRR 5284 and an invertase-deficient *Saccharomyces cerevisiae* 1403-7A. *Bioresour. Bioprocess.* **2021**, *8*, 85. [CrossRef]
144. Straathof, A.J.; Kieboom, A.P.; van Bekkum, H. Invertase-catalyzed fructosyl transfer in concentrated solutions of sucrose. *Carbohydr. Res.* **1986**, *146*, 154–159. [CrossRef] [PubMed]
145. Maugeri, F.; Hernalsteens, S. Screening of yeast strains for transfructosylating activity. *J. Mol. Catal. B Enzym.* **2007**, *49*, 43–49. [CrossRef]
146. Bhalla, T.C.; Thakur, N.; Thakur, N. Invertase of *Saccharomyces cerevisiae* SAA-612: Production, characterization, and application in synthesis of fructo-oligosaccharides. *LWT* **2017**, *77*, 178–185. [CrossRef]
147. Gomes, P.M.; Pereira, T.; Aparecida, C.; da Silva Santos, F.R.; Lisbo, N.F.; Fonseca, G.G.; Leite, R.S.R.; da Paz, M.F. Biochemical characterization, and evaluation of invertases produced from *Saccharomyces cerevisiae* CAT-1 and *Rhodotorula mucilaginosa* for the production of fructooligosaccharides. *Prep. Biochem. Biotechnol.* **2018**, *48*, 506–513. [CrossRef]
148. Zhang, J.; Liu, C.; Xie, Y.; Li, N.; Ning, Z.; Du, N.; Huang, X.; Zhong, Y. Enhancing fructooligosaccharides production by genetic improvement of the industrial fungus *Aspergillus niger* ATCC 20611. *J. Biotechnol.* **2017**, *249*, 25–33. [CrossRef]
149. Hidaka, H.; Hirayama, M.; Sumi, N. A fructooligosaccharide-producing enzyme from *Aspergillus niger* ATCC 20611. *Agric. Biol. Chem.* **1988**, *52*, 1181–1187. [CrossRef]
150. Nakakuki, T. Present status and future prospects of functional oligosaccharide development in Japan. *J. Appl. Glycosci.* **2005**, *52*, 267–271. [CrossRef]
151. Jung, K.H.; Bang, S.H.; Oh, T.K.; Park, H.J. Industrial production of fructooligosaccharides by immobilized cells of *Aureobasidium pullulans* in a packed bed reactor. *Biotechnol. Lett.* **2011**, *33*, 1621–1624. [CrossRef] [PubMed]
152. Han, S.; Pan, L.; Zeng, W.; Yang, L.; Yang, D.; Chen, G.; Liang, Z. Improved production of fructooligosaccharides (FOS) using a mutant strain of *Aspergillus oryzae* S719 overexpressing β-fructofuranosidase (FTase) genes. *LWT* **2021**, *146*, 111346. [CrossRef]
153. Mba, I.E.; Nweze, E.I. Nanoparticles as therapeutic options for treating multidrug-resistant bacteria: Research progress, challenges, and prospects. *World J. Microbiol. Biotechnol.* **2021**, *37*, 108. [CrossRef]
154. Kodym, A.; Afza, R. Physical and chemical mutagenesis. *Plant Funct. Genom.* **2003**, *236*, 189–203. [CrossRef]

155. Rajoka, M.I.; Yasmeen, A. Improved productivity of β-fructofuranosidase by a derepressed mutant of *Aspergillus niger* from conventional and non-conventional substrates. *World J. Microbiol. Biotechnol.* **2005**, *21*, 471–478. [CrossRef]
156. Lee, L.S.; Till, B.J.; Hill, H.; Huynh, O.A.; Jankowicz-Cieslak, J. Mutation and mutation screening. In *Cereal Genomics: Methods and Protocols*; Henry, R., Furtado, A., Eds.; Humana Press: Totowa, NJ, USA, 2014; Volume 1099, pp. 77–95. [CrossRef]
157. Terleth, C.; Schenk, P.; Poot, R.; Brouwer, J.; Van De Putte, P. Differential repair of UV damage in rad mutants of *Saccharomyces cerevisiae*: A possible function of G2 arrest upon UV irradiation. *Mol. Cell. Biol.* **1990**, *10*, 4678–4684. [CrossRef]
158. Witkin, E.M. Ultraviolet-induced mutation and DNA repair. *Annu. Rev. Genet.* **1969**, *3*, 525–552. [CrossRef]
159. Parekh, S.; Vinci, V.A.; Strobel, R.J. Improvement of microbial strains and fermentation processes. *Appl. Microbiol. Biotechnol.* **2000**, *54*, 287–301. [CrossRef]
160. Baeshen, N.A.; Baeshen, M.N.; Sheikh, A.; Bora, R.S.; Ahmed, M.M.M.; Ramadan, H.A.; Saini, K.S.; Redwan, E.M. Cell factories for insulin production. *Microb. Cell Factories* **2014**, *13*, 141. [CrossRef]
161. Lafraya, A.; Sanz-Aparicio, J.; Polaina, J.; Marín-Navarro, J. Fructo-oligosaccharide synthesis by mutant versions of *Saccharomyces cerevisiae* invertase. *Appl. Environ. Microbiol.* **2011**, *77*, 6148–6157. [CrossRef] [PubMed]
162. Schroeven, L.; Lammens, W.; Van Laere, A.; Van den Ende, W. Transforming wheat vacuolar invertase into a high affinity sucrose: Sucrose 1-fructosyltransferase. *New Phytol.* **2008**, *180*, 822–831. [CrossRef] [PubMed]
163. Yuan, X.L.; Goosen, C.; Kools, H.; van der Maarel, M.J.; van den Hondel, C.A.J.; Dijkhuizen, L.; Ram, A.F. Database mining and transcriptional analysis of genes encoding inulin-modifying enzymes of *Aspergillus Niger*. *Microbiol.* **2006**, *152*, 3061–3073. [CrossRef] [PubMed]
164. Chuankhayan, P.; Hsieh, C.Y.; Huang, Y.C.; Hsieh, Y.Y.; Guan, H.H.; Hsieh, Y.C.; Tien, Y.-C.; Chen, C.-D.; Chiang, C.-M.; Chen, C.-J. Crystal structures of *Aspergillus japonicus* fructosyltransferase complex with donor/acceptor substrates reveal complete subsites in the active site for catalysis. *J. Biol. Chem.* **2010**, *285*, 23251–23264. [CrossRef]
165. Jakob, F.; Meißner, D.; Vogel, R.F. Comparison of novel GH 68 levansucrases of levan-overproducing Gluconobacter species. *Acetic Acid Bact.* **2012**, *1*, e2. [CrossRef]
166. van Hijum, S.A.; Kralj, S.; Ozimek, L.K.; Dijkhuizen, L.; van Geel-Schutten, I.G. Structure-function relationships of glucansucrase and fructansucrase enzymes from lactic acid bacteria. *Microbiol. Mol. Biol. Rev.* **2006**, *70*, 157–176. [CrossRef]
167. Wuerges, J.; Caputi, L.; Cianci, M.; Boivin, S.; Meijers, R.; Benini, S. The crystal structure of *Erwinia amylovora* levansucrase provides a snapshot of the products of sucrose hydrolysis trapped into the active site. *J. Struct. Biol.* **2015**, *191*, 290–298. [CrossRef]
168. Kaplan-Türköz, B.; Bakar, B. Structural modelling and structure-function analysis of *Zymomonas mobilis* levansucrase. *J. Nat. Appl. Sci.* **2017**, *21*, 279–285. [CrossRef]
169. Martínez-Fleites, C.; Ortíz-Lombardía, M.; Pons, T.; Tarbouriech, N.; Taylor, E.J.; Arrieta, J.G.; Hernández, L.; Davies, G.J. Crystal structure of levansucrase from the Gram-negative bacterium *Gluconacetobacter diazotrophicus*. *Biochem. J.* **2005**, *390*, 19–27. [CrossRef]
170. Álvaro-Benito, M.; Polo, A.; González, B.; Fernández-Lobato, M.; Sanz-Aparicio, J. Structural and kinetic analysis of *Schwanniomyces occidentalis* invertase reveals a new oligomerization pattern and the role of its supplementary domain in substrate binding. *J. Biol. Chem.* **2010**, *285*, 13930–13941. [CrossRef] [PubMed]
171. Lanigan, T.M.; Kopera, H.C.; Saunders, T.L. Principles of genetic engineering. *Genes* **2020**, *11*, 291. [CrossRef] [PubMed]
172. Trollope, K.M. Engineering a Fungal β-Fructofuranosidase. Doctoral Thesis, Stellenbosch University, Stellenbosch, South Africa, 2015.
173. Stephanopoulos, G. Synthetic biology and metabolic engineering. *ACS Synth. Biol.* **2012**, *1*, 514–525. [CrossRef] [PubMed]
174. Xu, N.; Liu, Y.; Jiang, H.; Liu, J.; Ma, Y. Combining protein and metabolic engineering to construct efficient microbial cell factories. *Curr. Opin. Biotechnol.* **2020**, *66*, 27–35. [CrossRef]
175. Bailey, J.E. Toward a science of metabolic engineering. *Science* **1991**, *252*, 1668–1675. [CrossRef]
176. Benner, S.A.; Sismour, A.M. Synthetic biology. *Nat. Rev. Genet.* **2005**, *6*, 533–543. [CrossRef]
177. Martin, V.J.; Pitera, D.J.; Withers, S.T.; Newman, J.D.; Keasling, J.D. Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids. *Nat. Biotechnol.* **2003**, *21*, 796–802. [CrossRef]
178. Ke, J.; Yoshikuni, Y. Multi-chassis engineering for heterologous production of microbial natural products. *Curr. Opin. Biotechnol.* **2020**, *62*, 88–97. [CrossRef]
179. Medema, M.H.; Kottmann, R.; Yilmaz, P.; Cummings, M.; Biggins, J.B.; Blin, K.; de Bruijn, I.; Chooi, Y.H.; Claesen, J.; Coates, R.C.; et al. Minimum information about a biosynthetic gene cluster. *Nat. Chem. Biol.* **2015**, *11*, 625–631. [CrossRef]
180. Geers, A.U.; Buijs, Y.; Strube, M.L.; Gram, L.; Bentzon-Tilia, M. The natural product biosynthesis potential of the microbiomes of Earth–Bioprospecting for novel anti-microbial agents in the meta-omics era. *Comput. Struct. Biotechnol. J.* **2022**, *20*, 343–352. [CrossRef]
181. Adams, B.L. The next generation of synthetic biology chassis: Moving synthetic biology from the laboratory to the field. *ACS Synth. Biol.* **2016**, *5*, 1328–1330. [CrossRef] [PubMed]
182. Tian, T.; Kang, J.W.; Kang, A.; Lee, T.S. Redirecting metabolic flux via combinatorial multiplex CRISPRi-mediated repression for isopentenol production in *Escherichia coli*. *ACS Synth. Biol.* **2019**, *8*, 391–402. [CrossRef] [PubMed]
183. Kang, H.K.; Seo, M.Y.; Seo, E.S.; Kim, D.; Chung, S.Y.; Kimura, A.; Day, D.F.; Robyt, J.F. Cloning and expression of levansucrase from *Leuconostoc mesenteroides* B-512 FMC in *Escherichia coli*. *Biochim. Et Biophys. Acta (BBA)-Gene Struct. Expr.* **2005**, *1727*, 5–15. [CrossRef] [PubMed]

184. Braga, A.; Gomes, D.; Rainha, J.; Cardoso, B.B.; Amorim, C.; Silvério, S.C.; Fernández-Lobato, M.; Rodrigues, J.L.; Rodrigues, L.R. Tailoring fructooligosaccharides composition with engineered *Zymomonas mobilis* ZM4. *Appl. Microbiol. Biotechnol.* **2022**, *106*, 4617–4626. [CrossRef] [PubMed]
185. Chu, J.; Tian, Y.; Li, Q.; Liu, G.; Yu, Q.; Jiang, T.; He, B. Engineering the β-fructofuranosidase Fru6 with promoted transfructosylating capacity for fructooligosaccharide production. *J. Agric. Food Chem.* **2022**, *70*, 9694–9702. [CrossRef]
186. Yang, H.; Wang, Y.; Zhang, L.; Shen, W. Heterologous expression, and enzymatic characterization of fructosyltransferase from *Aspergillus niger* in *Pichia pastoris*. *New Biotechnol.* **2016**, *33*, 164–170. [CrossRef]
187. Aung, T.; Jiang, H.; Chen, C.C.; Liu, G.L.; Hu, Z.; Chi, Z.M.; Chi, Z. Production, gene cloning, and overexpression of a laccase in the marine-derived yeast *Aureobasidium melanogenum* strain 11-1 and characterization of the recombinant laccase. *Mar. Biotechnol.* **2019**, *21*, 76–87. [CrossRef]
188. Zhang, Y.; Zhang, X.; Xu, Y.; Xu, S.; Bilal, M.; Cheng, H. Engineering thermotolerant *Yarrowia lipolytica* for sustainable biosynthesis of mannitol and fructooligosaccharides. *Biochem. Eng. J.* **2022**, *187*, 108604. [CrossRef]
189. Franken, J.; Brandt, B.A.; Tai, S.L.; Bauer, F.F. Biosynthesis of levan, a bacterial extracellular polysaccharide, in the yeast *Saccharomyces cerevisiae*. *PLoS ONE* **2013**, *8*, e77499. [CrossRef]
190. Zhang, L.; An, J.; Li, L.; Wang, H.; Liu, D.; Li, N.; Cheng, H.; Deng, Z. Highly efficient fructooligosaccharides production by an erythritol-producing yeast *Yarrowia lipolytica* displaying fructosyltransferase. *J. Agric. Food Chem.* **2016**, *64*, 3828–3837. [CrossRef]
191. García, L.H.; Rodríguez, C.M.; Cruz, E.R.P.; García, D.M.; Lasa, A.M.; Ibañez, R.R.; Legon, A.S.; Toledo, L.E.T.; González, D.A. Novel enzymatic catalysts for fructooligosaccharides production from cane sugar. *Biotecnol. Apl.* **2015**, *32*, 1301–1307.
192. Burghardt, J.P.; Fan, R.; Baas, M.; Eckhardt, D.; Gerlach, D.; Czermak, P. Enhancing the heterologous fructosyltransferase activity of *Kluyveromyces lactis*: Developing a scaled-up process and abolishing invertase by CRISPR/Cas9 genome editing. *Front. Bioeng. Biotechnol.* **2020**, *8*, 607507. [CrossRef] [PubMed]

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