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Growth behavior of probiotic microorganisms on levan- and inulin-based fructans

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ABSTRACT

Modulation of the gut microbiota by prebiotics is an effective tool to improve host health. Here, the prebiotic properties of inulin- and levan-based fructans were investigated using microorganisms obtained from strain collections and isolated from probiotic pharmaceuticals and yogurts. Utilizing the recently characterized *endo*-levanase $LevB_{2286}$, levan-fructooligosaccharides (L-FOS) were produced and applied for dedicated growth studies. Real-time growth experiments in 48-well format revealed that 8 out of 17 strains isolated from probiotic products or yogurts responded to prebiotic treatment. *Lactobacillus paracasei* strains, several bifidobacteria, and a *Saccharomyces cerevisiae* isolate metabolized supplied fructans efficiently. Overall inulin-FOS were consumed more rapidly than L-FOS. However, this effect may be attributed to the lower average degree of polymerization and the presence of GF_n-FOS in the I-FOS preparation used. Growth experiments with fractionated L-FOS provided valuable insights into the influence of the degree of polymerization on fermentability by probiotic bacteria.

1. Introduction

With increasing knowledge about the diverse relationship between the intestinal microflora and human health, considerable interest in the modulation of the gut microbiota arose. Compounds that have been shown to be very effective in this context are prebiotics, which are defined as substrates that are selectively utilized by host microorganisms conferring a health benefit (Gibson et al., 2017). However, besides a long list of candidate prebiotics, only galactooligosaccharides (GOS), fructooligosaccharides (FOS), polymeric inulin, and the synthetic disaccharide lactulose meet the criteria necessary for the prebiotic classification to date (Scott et al., 2020). The beneficial properties of established prebiotics cover numerous physiological (Mohebbi et al., 2018; Mohebbi et al., 2019) and psychological functions (Ansari et al., 2020; Tabrizi et al., 2019), as well as physicochemical and sensory features (Beikzadeh et al., 2018), reinforcing their relevance for functional foods.

The health-promoting effects of the β-2,1-glycosidically linked fructan inulin and shorter-chain inulin-type FOS (I–FOS) were already emphasized during the introduction of the prebiotic concept (Gibson & Roberfroid, 1995). These effects were connected to the bifidogenic properties of inulin and I-FOS, which have been studied in numerous microbial growth experiments (Gibson & Roberfroid, 1995; Rossi et al., 2005). Today, inulin is commercially produced mainly from chicory and is widely used in the food sector as a prebiotic, fat replacer, and texture modifier (Shoaib et al., 2016). Due to its plant origin, commercial inulin has a comparably short chain length. The degree of polymerization (DP) from chicory inulin, for example, ranges from 2 to 60, with an average DP of 12 (Kelly, 2008, 2009). While plant fructans generally have a low DP range of 10 – 100, polyfructoses from bacterial origin can have high molecular masses with more than 10,000 fructose units (Öner et al., 2016; Shoaib et al., 2016).

Several studies indicate that the microbial fermentability of fructans depends on the DP of respective fructan preparations. Especially for the genus *Bifidobacterium*, but also for lactic acid bacteria, it was shown that the ability to degrade polymeric fructans is less widely spread than the ability to ferment FOS (DP < 10) (Falony et al., 2009; Rossi et al., 2005; Scott et al., 2014). In addition, better growth of bifidobacteria with short-chain FOS as well as a preferential consumption of FOS or fructans with a low DP was described in several studies (de Vuyst et al., 2014; Janer et al., 2004; Makras et al., 2005; McLaughlin et al., 2015).

Besides the DP, the presence of a terminal glucose residue also affects the fermentability of fructans (Wang et al., 2020). Since fructan synthesis is characterized by an initial fructosylation of sucrose, they often

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contain a terminal α–1,2 fructosyl-glucose linkage (Kelly, 2008, 2009). However, during the partial hydrolysis of polymeric fructans, FOS with (GF_n form) and without a terminal glucose residue (F_n form) arise. Based on growth experiments, Wang et al. (2020) revealed that bifidobacteria have a structural preference for the GF_n type.

Levan is one of the main fructan types and an alternative for the inulin-dominated fructan market. The fructan polymer predominantly consists of β-2,6-linked fructosyl units, and due to its unique physicochemical properties, levan has a wide field of applications (Öner et al., 2016; Srikanth et al., 2015). Besides its anti-oxidative, anti-inflammatory, and anti-microbial properties, levan exhibited a profound prebiotic effect. Several strains of the genus *Bifidobacterium* were shown to be able to grow on levan or L–FOS (Marx et al., 2000; Porras-Domínguez et al., 2014), and the recent study of Liu and colleagues using a three-stage continuous gut model system indicated an even stronger bifidogenic effect of levan compared to inulin (Liu et al., 2020). However, due to the rare commercial availability of levan and especially L-FOS, the data situation is still comparatively thin in this area.

An *endo*-levanase from *Azotobacter* (*A.*) *chroococcum* DSM 2286 was recently characterized, which cleaves polymeric levan into short-chain FOS with an extremely high specific activity (Hövels et al., 2021). The unique hydrolytic behavior of the enzyme was exploited in the present study to produce sufficient amounts of levan-based FOS. Plate reader-assisted growth experiments were performed to uncover the extent to which the linkage type and degree of polymerization of various fructans affect fermentability by probiotic representatives of the human gut. Using a total of 28 probiotic microorganisms, this work provides unique and comprehensive insights into the prebiotic properties of levan- and inulin-based fructans.

2. Material and methods

2.1. Materials

Chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, US) and Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Q5 High-fidelity DNA polymerase and PCR reagents were bought from New England Biolabs (Ipswich, US). Oligonucleotide primers were synthesized by Eurofins Scientific SE (Luxemburg, Luxemburg). Chicory inulin (DP 2–60) and the respective I-FOS (DP 2–8, generated by enzymatic hydrolysis of chicory inulin) were obtained from Megazyme Inc. (Bray, Ireland).

2.2. Enzymatic production of polymeric levan and levan-based FOS

Levan and L-FOS were enzymatically synthesized using the levansucrase $LevS_{1417}$ from *Gluconobacter japonicus* LMG 1417 (Hövels et al., 2020) and endo–levanase $LevB_{2286}$ from *A. chroococcum* DSM 2286 (Hövels et al., 2021). Production and purification of the recombinant enzymes were performed in *Escherichia* (*E.*) *coli* according to Hövels et al. (2020) and Hövels et al. (2021), respectively.

The processes described below for the production and purification of levan-based fructans are summarized in Fig. 1 in the form of a flowchart. For levan synthesis, 10 mM sodium acetate buffer (pH 5) and 100 μg $LevS_{1417}$ were added to 500 mL of a 2 M sucrose solution. The change in sucrose concentration was monitored by periodic sampling and subsequent HPLC analysis (see section 2.5). At a relative sucrose conversion of 90 %, the synthesized levan was precipitated with three parts of ethanol (96 % [v/v]). Following centrifugation (8000*g*, 5 min) and discarding of the resulting supernatant, the precipitate was resolved in 500 mL H_2O_{demin} and subjected to second precipitation under the same conditions. After final dissolution in 200 mL H_2O_{demin}, the precipitate was dialyzed against 10 L H_2O_{demin} (Nadir®–dialysis tube, Ø = 40 mm, MWCO ~ 10–20 kDa) and subsequently lyophilized. Lyophilization was performed using an Alpha 1–4 LCSplus freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The sample was exposed to a vacuum of 1 mbar at 20 °C for 48 h.

To hydrolyze the purified polymer into short-chain L-FOS, 100 mL of a 500 mM levan solution buffered with 5 mM McIlvaine buffer (pH 6) were supplemented with 100 μg recombinant $LevB_{2286}$. After four hours of incubation at 22 °C, the reaction was stopped by adding a threefold volume of ethanol (96 % [v/v]). After centrifugation of the mixture (11,000*g*, 10 min), ethanol was evaporated at 40 °C and 100 mbar in the rotational vacuum concentrator RVC2-25 CDplus (Martin Christ Gefriertrocknungsanlagen GmbH). Subsequent lyophilization was performed as described for the polymeric levan.

2.2.1. Acidic hydrolysis of carbohydrates utilized for growth experiments

To assess the structural composition of the utilized oligo- and polyfructoses, the substrates were subjected to acidic hydrolysis using a modified protocol according to Nguyen et al. (2009). 200 μl of 60 mM levan, inulin, L-FOS, I-FOS, and fructose solutions were mixed with 200 μl 2 % H_2SO_4 and heated at 100 °C for 30 min. After cooling down, 200 μl of the hydrolyzed substrates were neutralized with 80 μl 1 M NaOH. Released sugar monomers were quantified by HPLC as described in section 2.5.1.

2.3. Preparation of a probiotic strain collection

2.3.1. Microbial strains

Microbial strains (Table 1 were either purchased from the DSMZ - German Collection of Microorganisms and Cell Cultures GmbH (Braunschweig, Germany) or isolated from probiotic pharmaceuticals and yogurts.

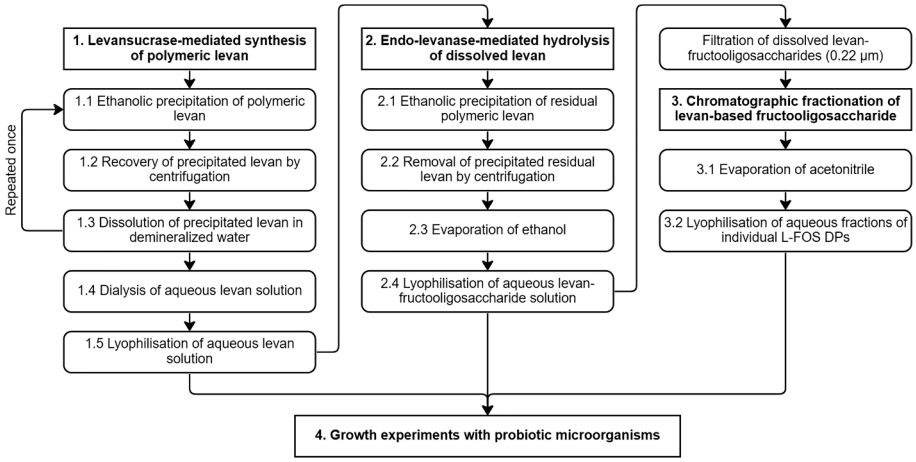


Fig. 1. Flowchart depicting the production and purification of levan-based fructans. The production of polymeric levan (1. – 1.5) was achieved using the recombinant levansucrase $LevS_{1417}$. Following the displayed downstream processing polymeric levan was hydrolyzed into levan-based FOS by the recombinant *endo*-levanase $LevB_{2286}$ (2. – 2.4). The obtained L-FOS mixture was fractionated by preparative HPLC and subjected to further downstream processing (3. – 3.2). In purified form, polymeric levan, the L-FOS mixture and individual L-FOS fractions were utilized for growth experiments with probiotic microorganisms (4.).

Table 1
Microbial strains utilized for growth experiments with levan- and inulin-type fructans.

Strain	Source	Distributer
Bifidobacterium adolescentis DSM 20083	Intestine of adult (Reuter 1963)	DSMZ GmbH
Bifidobacterium angulatum F16	Strain collection AG Deppenmeier	
Bifidobacterium breve DSM 20213	Intestine of infant (Reuter 1963)	DSMZ GmbH
Bifidobacterium longum subsp. *infantis* DSM 20088	Intestine of infant (Reuter 1963)	DSMZ GmbH
Bifidobacterium lactis JP12	SÖBBEKE ABC BIO Joghurt Natur	Molkerei Söbbeke GmbH (Gronau-Epe, Germany)
Bifidobacterium lactis JP13	Orthim Orthoflor® immun	orthim GmbH & Co. KG (Herzebrock-Clarholz, Germany)
Bifidobacterium animalis subsp. *lactis* JP14	Orthomol immun pro	Orthomol pharmazeutische Vertriebs GmbH (Langenfeld, Germany)
Bifidobacterium longum subsp. *longum* JP15	INBIOTYS Alflorex®	MEDICE Arzneimittel Pütter GmbH & Co. KG (Iserlohn, Germany)
Enterococcus faecium JP16	OMNiBiOTiC® 6	APG Allergosan Pharma GmbH (Graz, Austria)
Enterococcus faecium JP17	Nutrimmun® probiotik protect	nutrimmun GmbH (Münster, Germany)
Escherichia coli "Nissle 1917"	Provided by Prof. Dr. Galinski with the permission of Rudolf von Bünau	
Levilactobacillus brevis DSM 20054	Feces (Orla-Jensen 1919)	DSMZ GmbH
Lacticaseibacillus casei DSM 20011	Cheese (Orla-Jensen 1916)	DSMZ GmbH
Lacticaseibacillus casei JP3	Yakult®	Yakult Deutschland GmbH (Neuss, Germany)
Lacticaseibacillus casei JP4	SÖBBEKE ABC BIO Joghurt Natur	Molkerei Söbbeke GmbH (Gronau-Epe, Germany)
Lacticaseibacillus casei M57	Milking system	
Lacticaseibacillus paracasei DSM 20006	Beer (Collins et al., 1989)	DSMZ GmbH
Lacticaseibacillus paracasei JP5	APOSTELS jogurti natur (Greek yogurt)	APOSTEL Griechische Spezialitäten GmbH (Garbsen, Germany)
Lactiplantibacillus plantarum DSM 20174	Pickled cabbage (Orla-Jensen 1919)	DSMZ GmbH
Lactiplantibacillus plantarum JP8	Orthomol immun pro	Orthomol pharmazeutische Vertriebs GmbH (Langenfeld, Germany)
Lactiplantibacillus plantarum JP9	Nutrimmun® probiotik protect	nutrimmun GmbH (Münster, Germany)
Lacticaseibacillus rhamnosus JP6	Orthim Orthoflor® immun	orthim GmbH & Co. KG (Herzebrock-Clarholz, Germany)
Lacticaseibacillus rhamnosus JP7	Orthomol immun pro	Orthomol pharmazeutische Vertriebs GmbH (Langenfeld, Germany)
Lactococcus lactis JP1	OMNiBiOTiC® 6	APG Allergosan Pharma GmbH (Graz, Austria)
Lactococcus lactis JP2	ACTIVIA® Natur yogurt	DANONE GmbH (Haar, Germany)
Saccharomyces cerevisiae JP18	Perenterol® forte	MEDICE Arzneimittel Pütter GmbH & Co. KG (Iserlohn, Germany)
Streptococcus salivarius subsp. *thermophilus* DSM 20617	Pasteurized milk (Orla-Jensen 1919)	DSMZ GmbH
Streptococcus thermophilus JP11	APOSTELS jogurti natur (Greek yogurt)	APOSTEL Griechische Spezialitäten GmbH (Garbsen, Germany)

2.3.2. Isolation of probiotic strains

To generate a collection of probiotic microorganisms, material from probiotic products was streaked on brain heart infusion (BHI), de Man-Rogosa-Sharpe (MRS, (de MAN et al., 1960), without Tween 80), and soy peptone-yeast (30 g l^{-1} soy peptone, 3 g l^{-1} yeast extract) agar (1.5 % [w/v]) plates. The plates were incubated at 30 °C or 37 °C under aerobic, microaerophilic, or anoxic conditions. By repetitive streaking of single colonies, pure cultures were obtained. The identification of the isolated organisms was performed via BLAST analysis after amplification and sequencing of corresponding bacterial 16S rRNA (Table 2); BAC primers) or fungal rRNA gene (Table 2; ITS primers).

Identified species were cultivated in liquid medium under appropriate conditions for cryopreservation. The culture material of aerobic or microaerophilic species was mixed with sterile glycerol at a final concentration of 20 % [v/v] and stored at −70 °C. For anaerobic cryopreservation, sterile, anoxic sucrose solution which was thoroughly gassed with N_2 gas was added to the culture material at a final concentration of 20 % [w/v]. The solution was transferred to 20 mL-serum flasks and treated with N_2 gas prior storage at −70 °C.

2.4. Growth of probiotic microorganisms on levan- and inulin-based fructans

Except for the *Lactobacillus* species and *E. coli* "Nissle 1917″, all strains were cultivated in TPYM medium containing 2.5 g tryptone, 2.5 g peptone, 5 g yeast extract, 2.5 g meat extract, 2 g KH_2PO_4 and 40 mL salt solution (1 g l^{-1} K_2HPO_4, 1 g l^{-1} KH_2PO_4, 2 g l^{-1} NaCl, 10 g l^{-1} $NaHCO_3$, 0.25 g l^{-1} $CaCl_2 \times 2\ H_2O$, 0.5 g l^{-1} $MgSO_4 \times 7\ H_2O$) per liter H_2O_{demin}. Before inoculation 5 µg mL^{-1} hemin and 0.1 % [v/v] vitamin K1 were added to each culture as they are essential growth factors for various anaerobic microorganisms (Gibbons & Macdonald, 1960).

Lactobacilli were cultivated in a modified version of the carbohydrate-free MRS (cfMRS) medium (O'Donnell et al., 2011) containing 10 g peptone, 5 g yeast extract, 8 g Na-acetate × 3 H_2O, 2 g K_2HPO_4, 1.2 g NH_4Cl, 0.2 g $MgSO_4 \times 7\ H_2O$, and 0.06 g $MnCl_2 \times 4\ H_2O$. The pH was adjusted to 6.5 using HCl. *E. coli* "Nissle 1917″ was cultivated in M9 minimal medium (Miller, 1972). For the cultivation under anoxic conditions, the media were treated with N_2 gas, supplemented with 1 µg mL^{-1} resazurin as a redox indicator, and reduced with 0.5 mg mL^{-1} cysteine directly before use.

2.4.1. Precultures

To prepare precultures, the media described in section 2.4 were supplemented with 5 µg mL^{-1} glucose and inoculated with cell material from cryopreserved stocks. For aerobic cultivation, the precultures were incubated at 30 °C and 150 rpm in shake flasks. For anaerobic precultures, serum flasks under an N_2 / CO_2 (80 % / 20 %) atmosphere were inoculated from cryostocks and incubated at 37 °C without agitation.

2.4.2. Plate reader cultivation of probiotic microorganisms

Main cultures were cultivated in Greiner CELLSTAR® 48 well suspension culture plates with lid using a Tecan Infinite M200 plate reader (Tecan Group AG, Männedorf, Switzerland). Per well, 504 µl medium was mixed with 60 µl MES buffer (1 M, pH 6.5), 30 µl carbon source, and 6 µl preculture. Individual stock solutions of glucose, fructose, levan, inulin, L-FOS, and I-FOS were prepared at a concentration of 600 mM, resulting in final culture concentrations of 30 mM. For the oligo- and polysaccharides, the final concentration of 30 mM referred to the monomeric units. While oligosaccharide stocks were sterilized by filtration, polymeric stocks were autoclaved due to their high viscosity. To avoid acidic hydrolysis during autoclaving, levan and inulin stocks were supplemented with 5 mM potassium phosphate buffer (pH 7). Plate

Table 2
Oligonucleotide primers utilized for sequencing of bacterial and fungal rRNA.

Primer	Sequence 5′ − 3′	Reference
BAC338F	ACTCCTACGGGAGGCAG	(Yu et al., 2005)
BAC805R	GACTACCAGGGTATCTAATCC	
ITS1	TCCGTAGGTGAACCTGCGG	(White et al., 1990)
ITS4	TCCTCCGCTTATTGATATGC	

reader cultivations were performed at 30 or 37 °C, and in cycles of five minutes, the plates were linearly shaken for 2 min with an amplitude of 3 mm before OD-measurement at 600 nm (OD_{600}). After the stationary growth phase was reached, the cultivation was stopped, and the final pH was measured. For cultivation under anoxic conditions, the plate reader setup was introduced into an anoxic chamber (Coy Laboratory Products, Inc., Grass Lake, USA) and maintained in a CO_2 / N_2 / H_2 (49 % / 49 % / 2 %) atmosphere.

To compensate the backscatter effect of the OD_{600} measurement in undiluted cultures during plate reader cultivation, OD-calibration was performed for each microbial strain (except for strains of risk group 2). For this purpose, the OD_{600} values of several culture dilutions were measured in the plate reader setup and in appropriate dilution ($OD_{600} <$ 0.3) in a photometer. The OD_{600} values of the photometer were then plotted against the values of the plate reader, and the calibration equations were determined via quadratic regression through the zero point (Fig. S1).

2.5. Chromatographic methods

2.5.1. Qualitative and quantitative HPLC analysis

Chromatographic analysis was performed based on the HPLC setup used by Wienberg et al. (2021), which enabled the analysis of inulin-based FOS up to a DP of 17. Mono-, di-, and oligosaccharide samples were analyzed using a SpectraSYSTEM HPLC-system (Thermo Fisher Scientific Inc., Waltham, US) equipped with a degasser (SpectraSYSTEM SCM1000), a pump (SpectraSYSTEM P4000), an autosampler (SpectraSYSTEM AS3000) and a refraction index (RI) detector (RI-101, Ercatech AG, Bern, Switzerland). Sample separation was achieved using the main column Asahipak NH2P-50 4E, the guard column Asahipak NH2P-50G 4A, and an Asahipak NH2P-LF line filter (all Showa Denko Europe GmbH, Munich, Germany) at 40 °C. The mobile phase, 60 % [v/v] acetonitrile (MeCN), was applied in isocratic mode at a flow rate of 0.8 mL min^{-1}. To remove insoluble matter, samples were diluted with acetonitrile at a final concentration of 60 % [v/v] and centrifuged (11,000*g*, 1 min) before HPLC analysis. A consistent injection volume of 20 µl was ensured by an appropriate sample loop. Quantification was performed by the external standard method as described in Wienberg et al. (2021). FOS were quantified via the fructose standard.

2.5.2. Chromatographic fractionation of levan-FOS

Individual DPs of the prepared L-FOS mixture were fractionated by preparative HPLC (Fig. 1). Therefore, 1.62 g of the enzymatically produced L-FOS were solved in 50 mL 55 % MeCN and filtered through a PVDF-membrane with a pore size of 0.22 µm. Per run, 4.5 mL of this solution were injected into a preparative AZURA® HPLC-system (Knauer GmbH, Berlin, Germany), which consisted of the high-pressure-gradient pump P 6.1L, the preparative autosampler AS 6.1L equipped with a 10 mL sample loop, and the preparative refractive index detector RID 2.1L. Fractionation was achieved by the 16-port MultiPos Valve V 4.1 controlled by the valve drive VU 4.1. Data evaluation and automated handling of the valve drive were accomplished by ClarityChrom® 8.2.3 (Knauer GmbH) using thresholds of $<$ 15 mV (solvent recycling), $\geq$ 15 – 40 (waste), and greater than 40 (fraction collection). Separation was performed using the main column Eurospher II 100–5 NH2 250x20mm (Knauer GmbH) combined with the pre-column Eurospher II 100–5 NH2 50x20mm at 22 °C. The volumes of the eluent components acetonitrile and water were measured by weighing, based on the corresponding densities of 0.99 g cm^{-3} (water) and 0.79 g cm^{-3} (acetonitrile). The mobile phase, 55 % MeCN, was applied in isocratic mode at a flow rate of 20 mL min^{-1}. Volatile solvent components were removed from the obtained fractions using the rotary vacuum concentrator RVC 2–25 CDplus (Martin Christ Gefriertrocknungsanlagen GmbH). The samples were exposed to a vacuum of 75 mbar and 40 °C for six hours. The remaining aqueous solution was sterile filtered (PVDF-membrane; 0.22 µm) and lyophilized by freeze-drying (Alpha 1–4 LSCplus; Martin Christ Gefriertrocknungsanlagen GmbH). For this purpose, samples were subjected to a vacuum of 1 mbar at 20 °C for 24 h. Subsequently, bound water was removed at a vacuum of 0.01 mbar and a temperature of 30 °C for 2 h.

3. Results

3.1. Enzymatic production of polymeric levan and levan-type FOS

Sufficient amounts of polymeric levan were produced from sucrose using the recombinant enzyme $LevS_{1417}$ from *G. japonicus* LMG 1417 (Hövels et al. 2020). HPLC analyses of the purified levan revealed that small amounts of oligosaccharides (10 % [w/w]) and mono- and disaccharides (3 % [w/v], produced during the enzymatic conversion of sucrose were entrained during the purification process. Thus, the purity of the levan preparation was calculated to be 87 % [w/w].

Due to the unique hydrolytic properties of the *endo*-levanase $LevB_{2286}$ (Hövels et al., 2021), a fraction of the polymer was degraded efficiently into an L-FOS mixture (Fig. 2A). The monosaccharide content in the produced L-FOS was about 4 %, as determined by HPLC.

3.2. Analysis of levan- and inulin-based FOS utilized for growth experiments

L-FOS and I-FOS utilized for growth experiments were analyzed by HPLC to uncover differences in their structural composition besides their individual linkage type. Based on the manufacturer's specifications, the commercial I-FOS mixture should consist of FOS ranging from DP 2 to 8. A similar DP range was expected for the L-FOS mixture generated by recombinant $LevB_{2286}$ (Hövels et al., 2021). However, chromatographic analysis of the two oligosaccharide preparations revealed distinct differences regarding structural composition. The L-FOS had an average DP of 4.5 and showed the expected DP spectrum of 2 – 8 (Fig. 2A). The hexasaccharide levanhexaose was the major product, accounting for 32 % of the FOS generated.

In contrast to the manufacturer's specifications, DP8 was not detectable in the commercial I-FOS mixture (Fig. 2B). With DP3 and DP4 being predominant, the I-FOS mixture showed an average DP of 3.5. Thus, the I-FOS preparation showed a higher proportion of short-chain FOS compared to the L-FOS mixture (Fig. 2C). In addition, the obtained chromatograms (Fig. 2A + 2B) revealed a more heterogeneous composition of the commercial I-FOS mixture indicating relevant amounts of both F_n- and GF_n-FOS. The presence of higher amounts of GF_n-FOS in the commercial I-FOS was confirmed by acidic hydrolysis, revealing a substantially higher glucose content (8.7 %) compared to the L-FOS preparation (1.3 %). Chromatographic analysis and the acidic hydrolysis confirmed that $LevB_{2286}$-mediated cleavage of polymeric levan resulted in the formation of mostly F_n-type-FOS.

3.3. Growth of probiotic bacteria on levan- and inulin-based fructans

3.3.1. Strain isolation, method development and calibration of growth curves

A total of 17 pure cultures were isolated from probiotic pharmaceuticals and yogurts, most of which were lactic acid bacteria (Table 1). Together with probiotic isolates obtained from established strain collections, a total of 28 strains were thus investigated with respect to the ability to degrade various fructans. The backscatter effect arising from increased cell densities was compensated by calibrating the undiluted, online-measured OD_{600} values of the plate reader setup with appropriate dilutions measured in a benchtop-photometer (Tab. S1). With an average coefficient of determination of 0.997, the calibrations offered a valid tool to normalize the OD_{600} values obtained by the plate reader setup (Tab. S1). Comparing the growth curves in calibrated and non-calibrated form, the calibrated curves showed a clearly higher OD_{600} maximum (Fig. S2).

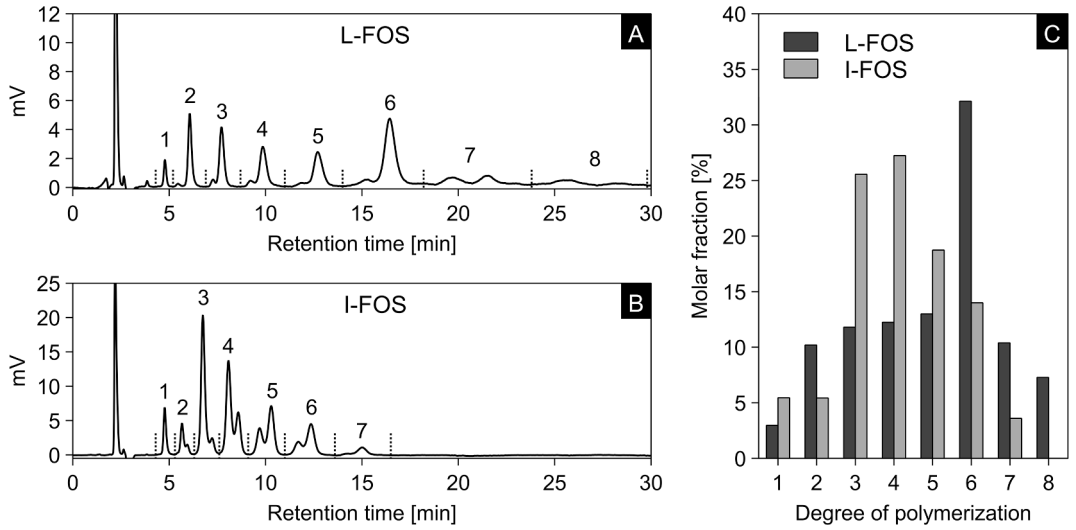


Fig. 2. Chromatographic analysis of L-FOS and I-FOS preparations utilized for growth experiments. HPLC chromatograms of L-FOS (**A**) and I–FOS (Megazyme Inc.) (**B**). 15 mM of the two FOS-mixtures were separated via HPLC. The mobile phase, 60 % [v/v] acetonitrile, was applied in isocratic mode at a flow rate of 0.8 mL min^{-1}. The peaks of the different DPs are separated by dashed lines and labeled according to their DP. **C**: Percentage of individual DPs in the total amount of L-FOS or I-FOS.

3.3.2. Growth of Lactobacillus sp. on levan- and inulin-type fructans

All investigated lactobacilli were successfully cultured on at least one carbon source at microplate scale (Fig. 3). Deviations between the biological replicates in terms of optical density and final pH value were very small, highlighting the functionality and overall performance of the plate reader cultivation. The detected OD_{600} values correlated inversely with the final pH, as microbial growth was accompanied by increased accumulation of acidic metabolic end products. Under aerobic conditions, the investigated lactobacilli utilized glucose rapidly, reaching optical densities ranging from 0.29 to 0.97 (Mean 0.73 ± 0.18). The metabolic end products lowered the pH from the initial value of 6.5 down to 5.54 ± 0.2.

Except for *L. brevis* DSM 20054 and *L. plantarum* DSM 20174, all lactobacilli could utilize fructose at an average maximal OD_{600} of 0.6 ± 0.21. The pH in respective cultures decreased from 6.5 to 5.47 ± 0.1. Besides the monomeric carbon sources, no other substrates were hydrolyzed efficiently by the investigated lactobacilli under aerobic conditions. Surprisingly, the ability of lactobacilli to metabolize fructans appeared to be dependent on the presence or absence of oxygen. Although *L. plantarum* DSM 20174 and *L. casei* DSM 20011 showed similar biomass formation and medium acidification in oxic and anoxic environments, *L. paracasei* strains utilized some fructans exclusively under anaerobic conditions. In the absence of oxygen, *L. paracasei* DSM 20006 and *L. paracasei* JP5 efficiently degraded L-FOS, I-FOS, and levan, whereas inulin was not metabolized. Maximal OD_{600} values obtained upon fructan degradation were comparable to the biomass yields achieved from the breakdown of monomeric substrates, indicating complete metabolization of the respective fructans (Fig. 3). However, monosaccharides were consumed more rapidly than oligomeric or polymeric substrates (Fig. 4).

Cultures supplemented with L-FOS, I-FOS, and polymeric levan showed initial growth on complex media components and then entered a second growth phase. In case of *L. paracasei* DSM 20006, the second growth phase was characterized by an initially very slow growth that increased exponentially and transitioned to a stationary plateau after 40 h of incubation. *L. paracasei* JP5 displayed a similar growth behavior, although the second growth phase showed a shorter lag phase and higher growth rate.

3.3.3. Growth of Lactococcus sp., Enterococcus sp., and Streptococcus sp. on levan- and inulin-type fructans

Except for *Lactococcus* (*Lc.*) *lactis* JP1 all investigated *Lactococcus*, *Enterococcus*, and *Streptococcus* strains could utilize the monosaccharides glucose and fructose under aerobic conditions (Fig. 3). Growth of *Lc. lactis* JP1 was restricted to glucose. The optical densities achieved upon glucose utilization were 1.04 ± 0.25 for *Lactococcus* sp., 0.57 ± 0.01 for *Enterococcus* sp., and 0.53 ± 0.09 for *Streptococcus* sp. Biomass yields achieved by *Enterococcus* and *Streptococcus* strains upon fructose consumption were comparable to those reached upon glucose consumption with maximal OD_{600} values of 0.57 ± 0.02 and 0.51 ± 0.1, respectively.

Of the *Lactococcus* strains examined, only *Lc. lactis* JP2 could metabolize fructose, resulting in an OD_{600} of 0.74 ± 0.02. While no consumption of levan- and inulin-type fructans was observed for the investigated *Lactococcus* and *Streptococcus* strains, *E. faecium* JP16 and *E. faecium* JP17 showed minor growth on levan- and inulin-type FOS. Utilization of L-FOS resulted in an optical density of 0.05 ± 0.03, while on I-FOS, a final OD_{600} of 0.15 ± 0.03 was measured for *Enterococcus* sp. None of the polymeric substrates was metabolized by the investigated cocci.

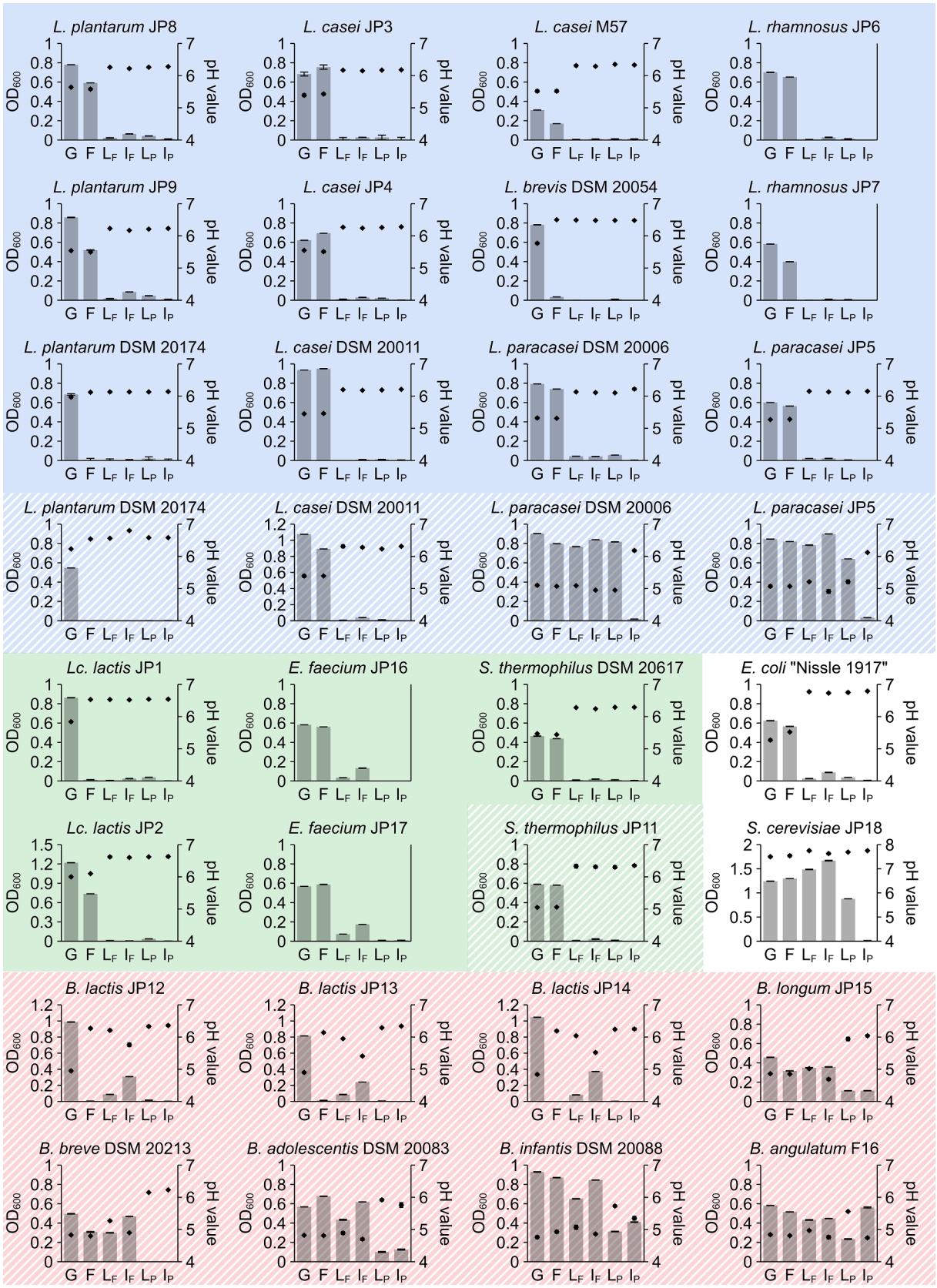
3.3.4. Growth of E. Coli "nissle 1917" and S. Cerevisiae JP18 on levan- and inulin-type fructans.

E. coli "Nissle 1917" showed elevated biomass yields in cultures supplemented with glucose and fructose, reaching optical densities of 0.62 ± 0.01 and 0.56 ± 0.01, respectively. No efficient degradation of levan- and inulin-type fructans was observed for the "Nissle" strain. The highest optical density in cultures supplemented with fructans was achieved upon I-FOS consumption (OD_{600}: 0.09 ± 0.01).

The isolated probiotic *S. cerevisiae* strain efficiently metabolized the monomeric substrates glucose and fructose, as well as L-FOS, I-FOS, and polymeric levan. No growth was detected in cultures supplemented with inulin. Consumption of glucose and fructose led to optical densities of 1.24 ± 0.01 and 1.3 ± 0.01, respectively. Upon the breakdown of polymeric levan, *S. cerevisiae* achieved a final OD_{600} of 0.88 ± 0.01. The highest optical densities were detected in wells containing L-FOS (1.48 ± 0.01) and I-FOS (1.67 ± 0.01), indicating complete consumption of the oligomeric substrates.

3.3.5. Growth of Bifidobacterium sp. on levan- and inulin-type fructans

In general, all bifidobacteria were able to metabolize glucose, and



(caption on next page)

Fig. 3. Maximum OD_{600} (■) and final pH (◆) values of cultivated *Lactobacillus* species (blue), other lactic acid bacteria (green), bifidobacteria (red), and other probiotic strains. Cultivations were performed in triplicates in a plate reader under oxic (solid background) or anoxic (striped background) conditions. Lactobacilli and *S. thermophilus* DSM 20617 were grown in the modified cfMRS medium. Bifidobacteria, other lactic acid bacteria, and *S. cerevisiae* JP18 were cultivated in TPYM medium. Growth of *E. coli* "Nissle 1917″ was investigated using M9 minimal medium. Anaerobic cultivations were performed at 37 °C. *L. paracasei*, *L. rhamnosus*, and *E. faecium* strains were also cultivated aerobically at 37 °C. All other aerobic cultivations were performed at 30 °C. Except for the cultures of *S. cerevisiae* JP18 and *E. coli* "Nissle 1917″, the respective medium was supplemented with 100 mM MES buffer (pH 6.5). All cultures were inoculated with 1 % [v/v] preculture. Glucose (G), fructose (F), L-FOS (L_F), I-FOS (I_F), levan (L_P), and inulin (I_P) were used as carbon sources and supplemented at a final concentration of 30 mM, referring to the monomeric units. In control cultures the carbon source was substituted with water. The maximum OD_{600} values of the control cultures were subtracted from the values of cultures containing a carbon source to exclude the growth on complex media components. The final pH of cultures of the R2 species (*L. rhamnosus* and *E. faecium*) could not be determined. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

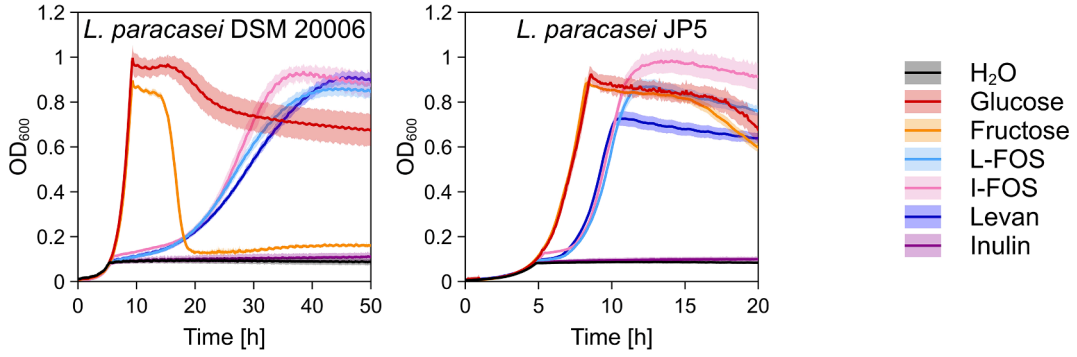


Fig. 4. Growth of *L. paracasei* DSM 20006 and *L. paracasei* JP5 on levan- and inulin-based fructans. Cultivation was performed in biological triplicates in a plate reader under anoxic conditions at 37 °C. The modified cfMRS medium supplemented with 100 mM MES buffer (pH 6.5) and 30 mM substrate was inoculated with 1 % [v/v] preculture. OD_{600} was measured every five minutes. The dark, central line of the growth curves reflects the mean of each biological triplicate, while the lighter area above and below it corresponds to the standard deviation.

except for the *B. lactis* strains, growth was also observed on fructose (Fig. 3). The optical densities achieved upon utilization of glucose and fructose varied from 0.46 to 1.05 (mean: 0.73 ± 0.24) and 0.31 to 0.87 (mean: 0.54 ± 0.24), respectively. Of the genera studied, the genus *Bifidobacterium* was the most capable regarding fructan consumption. All cultured bifidobacteria could utilize oligomeric L- and I-FOS; however, the degree of metabolization varied substantially (Figs. 3 & 4). Optical densities in wells supplemented with L-FOS ranged from 0.08 ± 0.01 (*B. lactis* JP14) to 0.65 ± 0.03 (*B. infantis* DSM 20088) with an average OD_{600} of 0.32 ± 0.19. I–FOS consumption led to optical densities ranging from 0.24 ± 0.01 (*B. lactis* JP13) to 0.85 ± 0.01 (*B. infantis* DSM 20088) with an average OD_{600} of 0.46 ± 0.19. The growth of *B. longum* JP15, *B. breve* DSM 20213, and *B. adolescentis* DSM 20083 on L-FOS was characterized by a distinct, biphasic growth pattern (Fig. 5). In contrast, utilization of I-FOS resulted in more uniform growth curves for the respective organisms. Growth on polymeric levan and inulin was observed for *B. longum* JP15, *B. adolescentis* DSM 20083, *B. infantis* DSM 20088, and *B. angulatum* F16. Overall, inulin was consumed more efficiently by these four strains (OD_{600}: 0.3 ± 0.22) than levan (OD_{600}: 0.19 ± 0.1).

3.4. Growth of probiotic bacteria on fractionated levan-type FOS

3.4.1. Purification of individual L-FOS fractions for growth experiments

To investigate the effect of the degree of polymerization of L-FOS on the digestibility by probiotic bacteria, the prepared L-FOS mixture was fractionated by preparative HPLC. The F_n-type FOS present in the L-FOS-mixture were successfully separated by the chromatographic setup (Fig. 6). Based on the detected peak areas, an average purity of 94.4 ± 6.6 % was determined for the dissolved fractions (Fig, S3). The reduced purity was caused by co-fractionation of adjacent peaks. This effect was most remarkable for levanhexaose (DP6), where co-fractionation of the DPs 4 and 5 decreased the purity to 82.7 %.

Concentration of the redissolved fractions was quantified based on peak area in relation to levanpentaose (DP5) since the corresponding peak area could be considered the distribution's mean value. For this purpose, the total peak area of DP5 was set to 100 % and the total peak areas of the other DPs were proportioned accordingly. In general, no large variations were observed between the different preparations. In comparison to levanpentaose, levanhexaose showed the lowest relative concentration at 91.7 %, whereas levantetraose (DP4) had the highest relative concentration at 105.5 % (Fig. S3).

3.4.2. Plate-reader cultivation of Bifidobacterium sp. and L. paracasei DSM 20006 supplemented with fractionated L-FOS

During the initial growth experiments, a distinct, biphasic growth behavior was observed for some probiotic strains capable of metabolizing the supplemented L-FOS mixture (Fig. 4 + 5). This behavior indicated that the different degrees of polymerization were taken up and/or hydrolyzed to different extents. To clarify this observation, a total of four probiotic strains were cultivated with L–FOS of individual DPs, fractionated by preparative HPLC. *B. adolescentis* DSM 20083 utilized glucose, levanbiose (DP2), and levantriose (DP3) rapidly, reaching optical densities of 0.82 ± 0.01, 0.89 ± 0.01, and 0.87 ± 0.01, respectively (Fig. 7). Cultures supplemented with levantetraose entered a growth plateau after consumption of complex media components, in which almost no increase in biomass was observable. Growth again increased exponentially from this plateau, leading to a maximal optical density of 0.64 ± after 25 h of incubation. Growth behavior on levanpentaose was quite similar. However, the growth plateau was much longer, whereby the final optical density of 0.59 ± 0.01 was reached just before the end of the 40-hours experiment. Maximal doubling times (t_d) on DP4 (230 mins) and DP5 (213 mins) were considerably longer compared to DP2 (50 mins), DP3 (51 mins), and glucose (60 mins). In contrast to the DPs 2 to 5, DP6 could not be utilized by *B. adolescentis* DSM 20083. Cultures supplemented with the L-FOS mixture showed a rapid increase in biomass up to an optical density of 0.28. Afterward, growth transitioned into a plateau with a slow increase in biomass towards the end of the experiment. Upon L-FOS utilization, a final OD_{600} of 0.43 ± 0.01 was achieved.

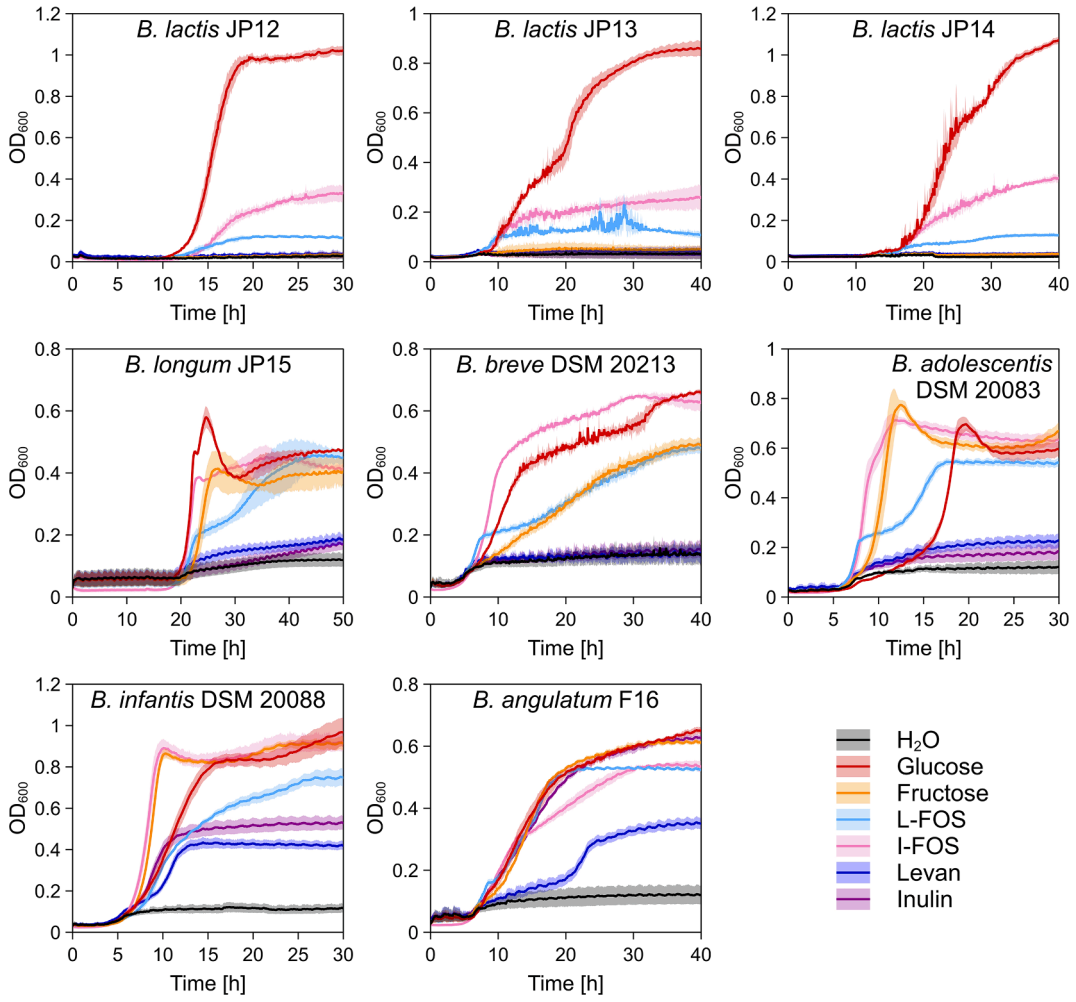


Fig. 5. Growth of bifidobacteria on levan- and inulin-based fructans. Cultivation was performed in triplicates in a plate reader under anoxic conditions at 37 °C. TPYM medium supplemented with 100 mM MES buffer (pH 6.5) and 30 mM substrate was inoculated with 1 % [v/v] preculture. OD_{600} was measured every five minutes. The dark, central line of the growth curves reflects the mean of each biological triplicate, while the lighter area above and below it corresponds to the standard deviation.

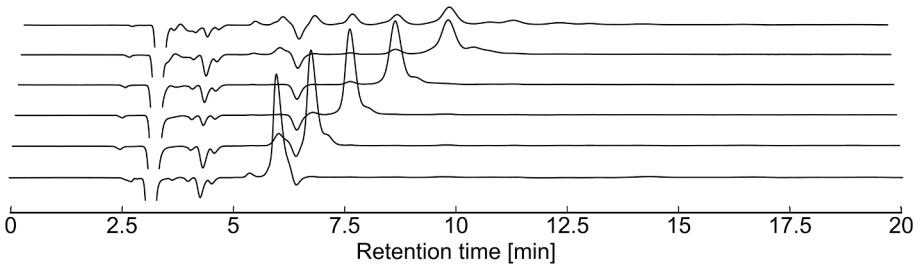


Fig. 6. HPLC chromatograms of an initial L-FOS mixture and individual DPs isolated by preparative HPLC. The peaks of the different fractions are labeled according to their DP. The L-FOS mixture was separated by preparative HPLC using an amino phase column. Isolated fractions as well as the initial L-FOS mixture were subsequently analyzed by HPLC using the Asahipak column setup (section 2.4.1) and 65 % [v/v] acetonitrile at a flow rate of 0.6 mL min^{-1}.

The growth behavior of *B. breve* DSM 20213 was comparable to that of *B. adolescentis* DSM 20083 (Fig. 7). Glucose, levanbiose, and levantriose were consumed rapidly, leading to optical densities of 0.72 ± 0.01, 0.64 ± 0.06, and 0.66 ± 0.03, respectively. Maximum doubling times on the respective substrates were 64 min (glucose), 48 min (DP2), and 47 min (DP3). The metabolization of levantetraose led to a linear growth at a doubling time of 278 min, resulting in a final OD_{600} of 0.63 ± 0.02 after 25 h of incubation. Cultures supplemented with levanpentaose showed a growth plateau after consumption of complex media components. However, the plateau returned to exponential growth (t_d = 227 min), whereby an optical density of 0.5 ± 0.02 was measured just before the end of the experiment. Again, no growth was detected on levanhexaose. L-FOS utilization was characterized by a rapid increase in biomass during the early growth phase, followed by a second growth phase with a linear elevation in biomass. After 40 h of incubation, a final OD_{600} of 0.42 ± 0.03 was measured in wells supplemented with L-FOS.

B. infantis DSM 20088 showed the best overall ability to utilize various DPs of levan-type FOS (Fig. 7). In contrast to *B. adolescentis* DSM 20083 and *B. breve* DSM 20213, *B. infantis* DSM 20088 rapidly consumed all substrates without forming temporary growth plateaus. The

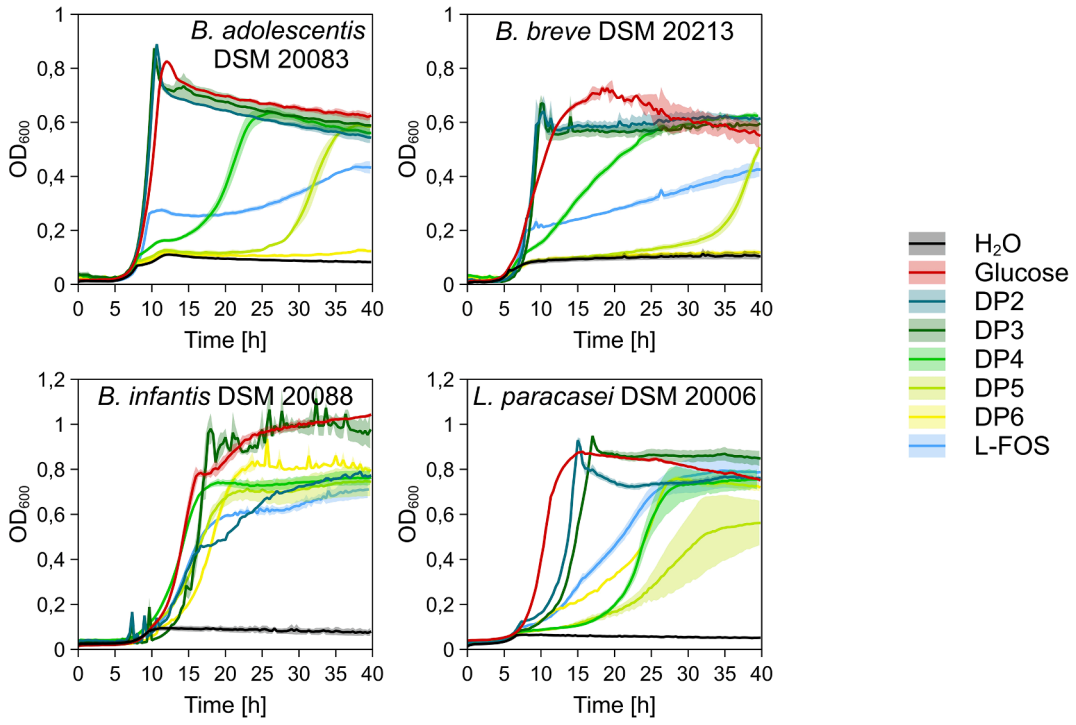


Fig. 7. Growth of probiotic bacteria on fractionated L-FOS. Cultivation was performed in triplicates in a plate reader under anoxic conditions at 37 °C. TPYM medium (bifidobacteria) or the modified cfMRS medium (*L. paracasei*) supplemented with 100 mM MES buffer (pH 6.5) and 30 mM substrate was inoculated with 1 % [v/v] preculture. OD_{600} was measured every five minutes. The dark, central line of the growth curves reflects the mean of each biological triplicate, while the lighter area above and below it corresponds to the standard deviation.

stationary phase was partially characterized by strong fluctuations of the optical density, which hindered a valid comparison of the maximal OD_{600} values. Nevertheless, it was evident that an optical density of at least 0.6 was achieved on all substances within 22 h of incubation. During the exponential growth phase, doubling times of 109 min (glucose), 127 min (DP2), 110 min (DP3), 138 min (DP4), 188 min (DP5), 184 min (DP6), and 139 min (L-FOS mixture) were assessed.

The growth rates of *L. paracasei* DSM 20006 were again strongly dependent on the degree of polymerization of the oligosaccharides supplied (Fig. 7). While cultures supplemented with glucose showed a clean exponential growth curve, all other cultures exhibited decreased growth after complex media components were consumed. Afterward, biomass formation increased with doubling times of 113 min (glucose), 109 min (DP2), 130 min (DP3), 184 min (DP4), 408 min (DP5), and 419 min (DP6). Utilization of the L-FOS mixture was characterized by moderate growth on short-chain FOS (t_d = 248 mins) followed by slow growth on long-chain FOS with a doubling time of 477 min. Except for cultures supplemented with DP5, all wells treated with a carbon source reached comparable maximal optical densities (mean: 0.84 ± 0.09) ranging from 0.76 ± 0.1 (DP4) to 0.95 ± 0.03 (DP3), indicating complete breakdown of corresponding L-FOS fractions. Upon levanpentaose consumption a final OD_{600} of 0.56 ± 0.17 was detected at the end of the 40-hour experiment.

4. Discussion

The work carried out was intended to shed light on the prebiotic properties of levan-type FOS and to reveal the extent to which their degree of polymerization affects the growth of probiotic bacteria.

The recently characterized enzymes $LevS_{1417}$ (Hövels et al., 2020) and $LevB_{2286}$ (Hövels et al., 2021) enabled rapid production of polymeric levan and L-FOS (Fig. 2A). Chromatographic analyses revealed that the synthesized L-FOS were almost exclusively F_n-type FOS, whereas the commercial I-FOS mixture contained relevant amounts of GF_n-FOS (Fig. 2B). The appearance of F_n-FOS and GF_n-FOS was already described for I-FOS mixtures generated by enzymatic hydrolysis of inulin (Roberfroid, 2007). The chromatographic analyses further revealed that L-FOS were retarded more strongly by the amino phase column than I-FOS, suggesting that the β-2,6-glycosidic linkage has a reinforcing effect on the polar character of fructooligosaccharides.

Based on a total of 28 microbial strains supplied from strain collections or isolated from probiotic pharmaceuticals or yogurts, the automated plate reader setup provided detailed insights into the fermentability of inulin- and levan-based fructans by probiotic bacteria (Figs. 3 – 5, 7). Similar approaches for monitoring bacterial growth have been described in the literature (Cernat & Scott, 2012; Scott et al., 2014). Experiments comparing the growth of different bacterial species in 96-well plates with standard cultivation in Hungate tubes showed good agreement between both methods. However, said studies reported slightly lower maximum OD_{600} values and shorter exponential growth phases using microtiter cultivation (Cernat & Scott, 2012; Scott et al., 2014). The backscatter effect of dense cell suspensions, which was probably the trigger for these issues, was compensated by individual calibrations in the study presented here. Furthermore, it was found that both a reduction of the well number from 96 to 48 and the change from orbital to linear shaking mode had a positive effect on the progression of the growth curves (not shown).

While most lactobacilli studies showed no growth on fructans, *L. paracasei* strains displayed broad structural adaptability towards levan- and inulin-type fructans (Fig. 3). However, this observation was limited to cultures maintained under anoxic conditions, as *L. paracasei* strains could not utilize fructans under oxic conditions. The frequent ability of *L. paracasei* strains to ferment fructans has already been reported (Makras et al., 2005; Müller & Lier, 1994; Porras-Domínguez et al., 2014; Renye et al., 2021; Wang et al., 2020). Müller and Lier revealed that from a high number of lactic acid bacteria, all *L. paracasei* strains utilized polyfructoses for growth. In addition, the researchers found that *L. paracasei* strains efficiently metabolized plant-derived

levan, whereas only half of them was able to degrade inulin (Müller & Lier, 1994). These observations are consistent with the results obtained here. *L. paracasei* strains, originating from completely different sources (Table 1), utilized levan but not inulin. These results are further in accordance with studies showing that the cell-wall anchored β–fructofuranosidase of *L. paracasei* had a higher affinity for bacterial levan than for chicory inulin (Martel et al., 2010) and that the extracellular β-fructofuranosidase hydrolyzed β-2,6-linked fructan more rapidly than the β-2,1 linkages of inulin (Müller & Seyfarth, 1997). Nevertheless, the β–fructofuranosidases of *L. paracasei* are generally able to hydrolyze levan- as well as inulin-type fructans (Martel et al., 2010; Müller & Seyfarth, 1997; Yong et al., 2007), which was confirmed by the fermentation of L- and I–FOS in the present study.

Although inulin was proposed as a promising prebiotic inclusion for synbiotics containing *Lc. lactis* (Almutairi et al., 2021), the *Lc. lactis* strains investigated in this work showed no growth on levan- or inulin-based fructans. These results reinforce the prevailing tendency in the literature that *Lc. lactis* is incapable of fructan degradation. For instance, Siezen and colleagues observed that several plant-derived *Lc. lactis* isolates were unable to efficiently utilize levan or inulin (Siezen et al., 2008).

The low growth of probiotic strains *E. faecium* JP16 and JP17 on short-chain fructans contradicts observations from the literature (Ayala Monter et al., 2018; Khosravi et al., 2018). Khosravi et al. revealed that *E. faecium* DSM 3530 possessed limited ability to degrade long-chain fructans but was quite efficient at metabolizing short-chain FOS with an average DP of 4. This degree of polymerization is consistent with the average chain length of the I-FOS mixture used in this work. This divergence may indicate that the ability of *E. faecium* to degrade fructans is strain-specific.

While no growth of *S. thermophilus* strains on fructans was observed in this study (Fig. 3), Oliveira et al. reported a shortened generation time for *S. thermophilus* TA040 cultured in skim milk supplemented with inulin (de Souza Oliveira et al., 2011). Thus, utilization of levan- and inulin-based fructans by *S. thermophilus* isolates also appears to be strain-dependent.

To the best of our knowledge, the growth of *E. coli* "Nissle 1917″ on fructans has not yet been studied. Based on the results obtained here, it can be assumed that the "Nissle"-strain is incapable of degrading levan- and inulin-type fructans (Fig. 3).

In contrast to most lactic acid bacteria and *E. coli* "Nissle 1917″, the probiotic yeast *S. cerevisiae* showed remarkable growth not only on I-FOS, but also on L-FOS and levan (Fig. 3). To our knowledge, this work provides the first evidence that *S. cerevisiae* can efficiently metabolize levan-type fructans. It is known that the invertase SUC2, encoded in the genome of *S. cerevisiae* strain JZ1C, is capable of degrading β-2,1-glycosidic linkages between the fructosyl-units of inulin (Wang & Li, 2013). The gene product of an *SUC2* homolog may have catalyzed the observed fructan degradation. However, no degradation was observed for inulin, contradicting the findings in the literature.

The conducted growth experiments revealed that bifidobacteria were the most capable probiotic bacteria with regard to fructan degradation (Fig. 3). While the isolated *B. lactis* strains showed only minor growth on I-FOS and L-FOS all remaining bifidobacteria achieved elevated biomass upon growth on fructans. This observation is consistent with previous studies in the literature demonstrating that bifidobacteria are capable of fermenting inulin- and levan-type fructans in a strain- and species-dependent manner (Falony et al., 2009; Marx et al., 2000; Porras-Domínguez et al., 2014; Sakata et al., 2002). Except for *B. angulatum* F16, the bifidobacteria studied showed faster growth on I-FOS compared to L-FOS, indicating preferential uptake and/or hydrolysis of β-2,1-glycosidic linked fructosyl-moieties. However, as the chromatographic studies revealed, the FOS preparations differed in terms of polymerization degree and monosaccharide composition (Fig. 2). The higher average DP and the absence of GF_n-FOS could have caused the inferior growth on L-FOS. A recent study investigating the prebiotic effect of GF_n-FOS and H-FOS (mixture of GF_n-FOS and F_n-FOS) found that GF_n-FOS had superior bifidogenic properties than H-FOS (Wang et al., 2020). The poor growth of *B. lactis* strains on FOS and the inability to ferment fructose may indicate that the organisms fed exclusively on the terminal glucose moieties during FOS consumption. This theory would explain the higher final OD_{600} of the *B. lactis* strains on I-FOS, as a higher proportion of GF_n-FOS was detected in the I-FOS mixture. In the presence of L-FOS, several bifidobacteria displayed biphasic growth, indicating varying affinity towards the different DPs of the supplied FOS. Growth experiments based on fractionated L-FOS revealed, that the di- and trisaccharides levanbiose and levantriose were metabolized rapidly by selected probiotic bacteria. *B. adolescentis* DSM 20083 and *B. breve* 20213 achieved even higher growth rates on DP2 and DP3 than on glucose. These data support observations from the literature, according to which various bifidobacteria showed higher growth rates on di- and trisaccharides than on monomeric glucose (Rada et al., 2002). Furthermore, the suggestion of Marx et al. that the decisive factor for fructan degradation could be the molecular mass and not the linkage type of the fructan is confirmed here (Marx et al., 2000).

5. Conclusion

Fructans are important ingredients in the functional food industry due to their positive effects on the gut microbiota and associated physiological functions. In this study, an automated biomass detection system provided valuable insights into the degradability of various fructans by probiotic microorganisms under oxic and anoxic conditions. The experiments revealed that 8 out of 17 microbial strains isolated from probiotic pharmaceuticals or yogurts responded to prebiotic treatment with different fructan preparations. It was verified that inulin- and levan-based fructans can be efficiently metabolized by selected lactobacilli and numerous bifidobacteria. Remarkably, the probiotic yeast *S. cerevisiae* and *L. paracasei* strains, which showed no growth on inulin, were able to efficiently utilize polymeric levan. However, fructan degradation by the *L. paracasei* strains studied was limited to cultures grown in the absence of oxygen. Growth experiments with fractionated L-FOS confirmed observations from the literature that the degree of polymerization has a fundamental impact on the prebiotic effect of FOS and that short-chain L-FOS are consumed rapidly by bifidobacteria and *L. paracasei* DSM 20006. Thus, this work provides valuable information on the efficacy of different probiotic-prebiotic combinations for synbiotic formulations.

CRediT authorship contribution statement

Joana Charlot Pohlentz: Investigation, Methodology, Validation, Visualization, Writing – original draft. **Nicole Gallala:** Investigation, Writing – review & editing. **Konrad Kosciow:** Conceptualization, Supervision, Writing – review & editing. **Marcel Hövels:** Conceptualization, Methodology, Writing – original draft, Supervision, Project administration.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Ethics Statement

This study was conducted without human or animal subjects. The authors declare no ethical conflicts.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2022.105343.

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