

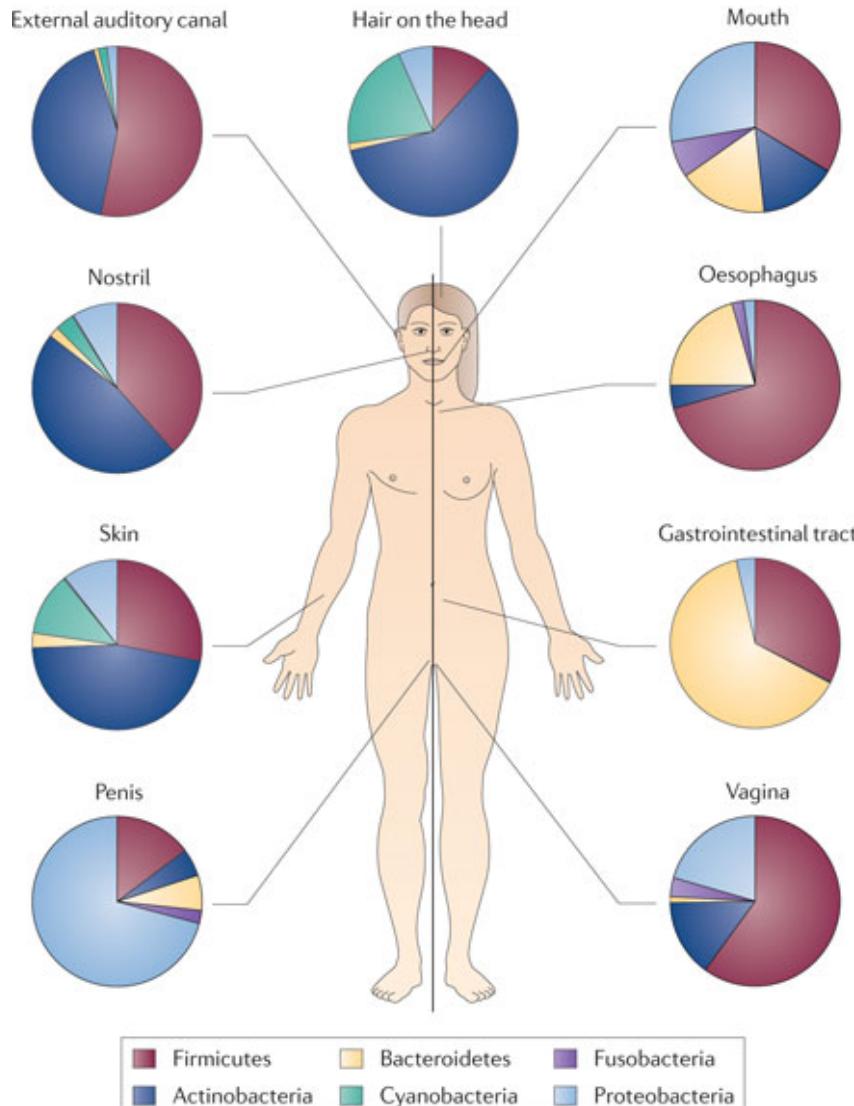


CCC3 Symposium, 30-11-2017

The role of selected probiotic bacteria in degradation
of (prebiotic) carbohydrates

Markus Böger, PhD student University of Groningen

Humans harbor diverse microbial communities



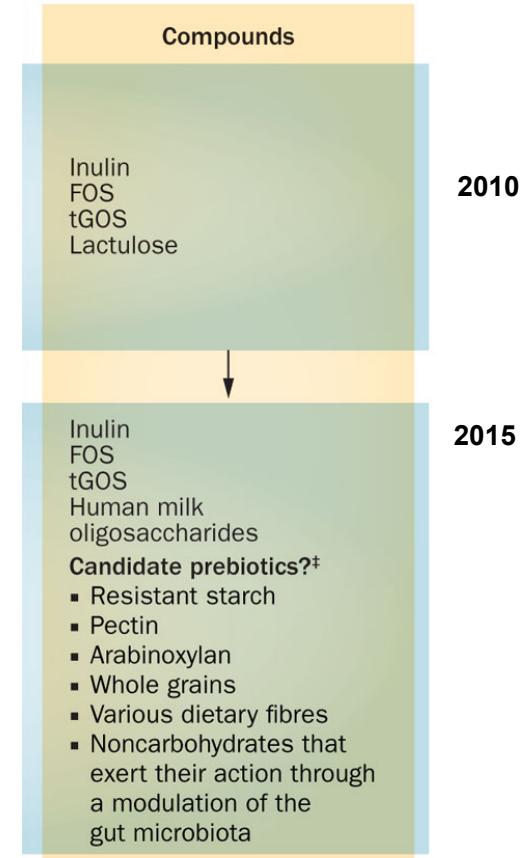
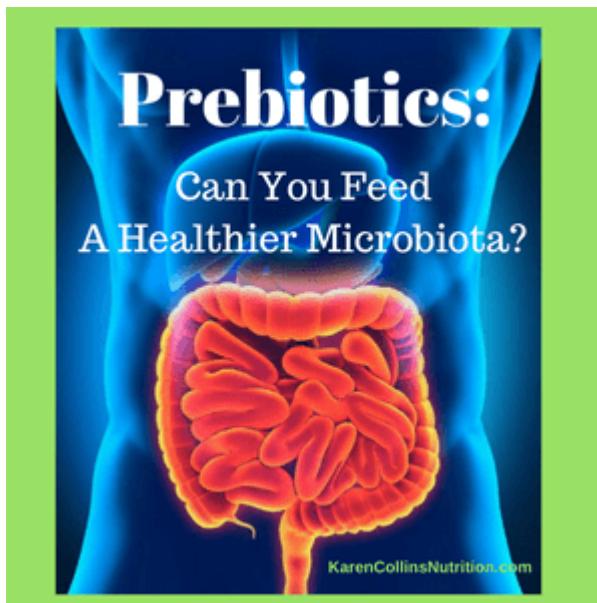
Blandino G et al. Diabetes Metab. 2016 NOV;42(5): 303-15

Probiotics: live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [1].

Synergy between pre- and probiotics?



Prebiotic: 'a substrate that is selectively utilized by host microorganisms conferring a health benefit' [2].



#Prebiotic candidates, needs additional research.

Bindels LB et al. Nat Rev Gastroenterol Hepatol. 2015 MAY;12(5):303-10

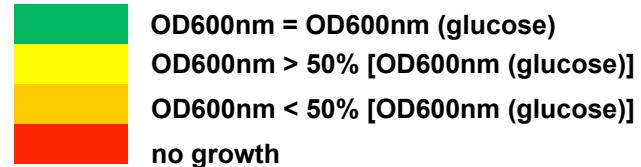
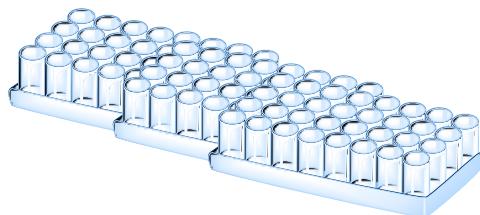
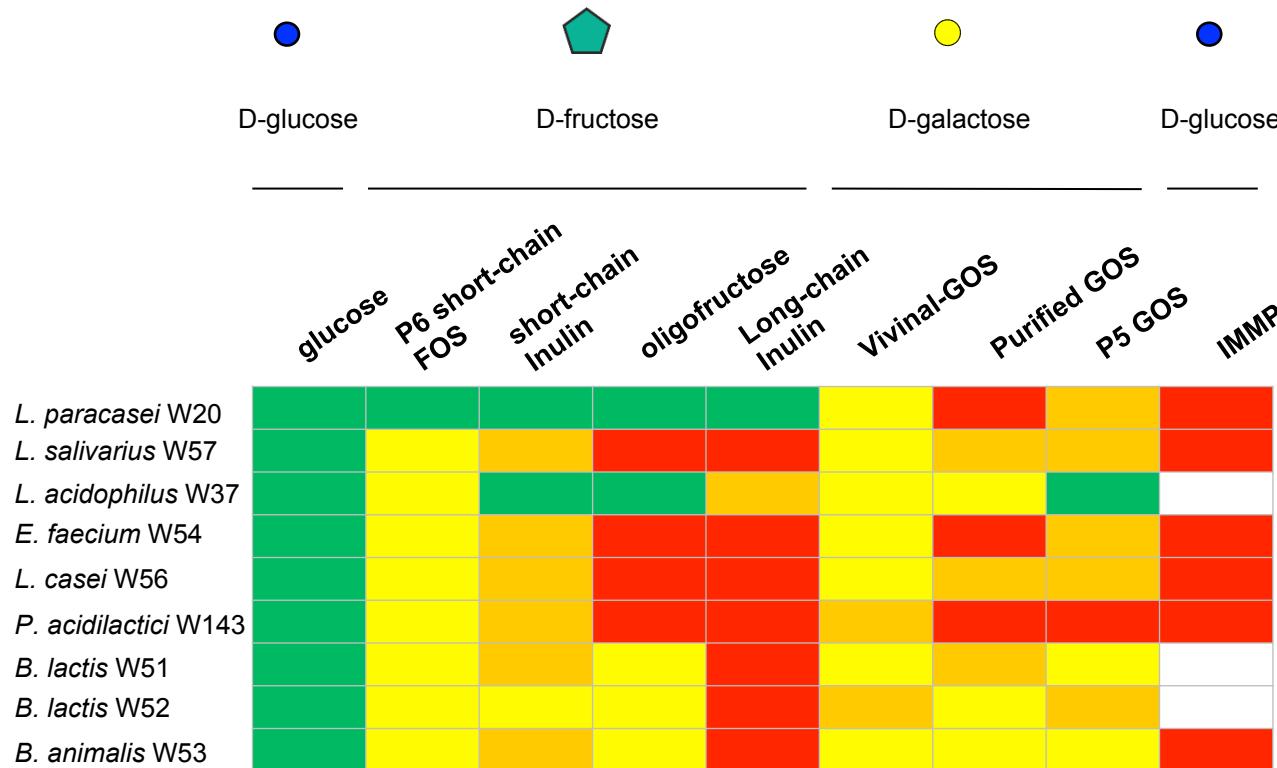
Objectives of the project



- Structural analysis of (prebiotic) carbohydrates prior to and after probiotic degradation
- Annotation of genes involved in carbohydrate metabolism of probiotic bacteria
- Anaerobic growth experiments to test suitability of carbohydrates for probiotic consumption
- Formulate new synbiotics based on metabolic pathways employed by probiotics for carbohydrate degradation

CCC3 Subproject 1:
Carbohydrate structural analysis
CCC3 Subproject 4:
Effects on the microbiota

Anaerobic screening of probiotic growth on carbohydrates

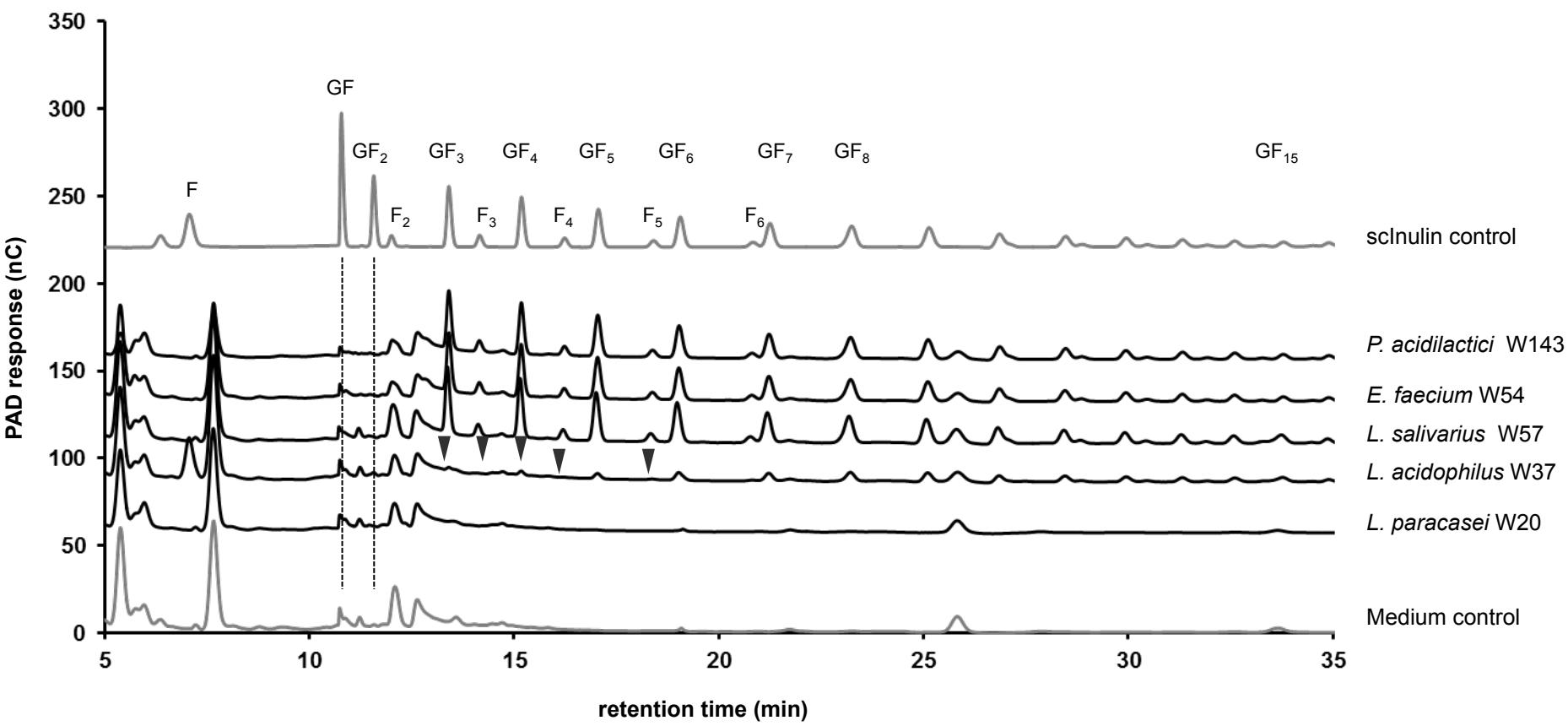


Reaction volume: 200 µl with 5 mg/ml carbohydrate

Consumption of (prebiotic) components from sInulin (Frutafit® CLR)



$G = D\text{-glucose}$
 $F = D\text{-fructose}$
 $n = 1 - 40 \text{ in } sInulin$

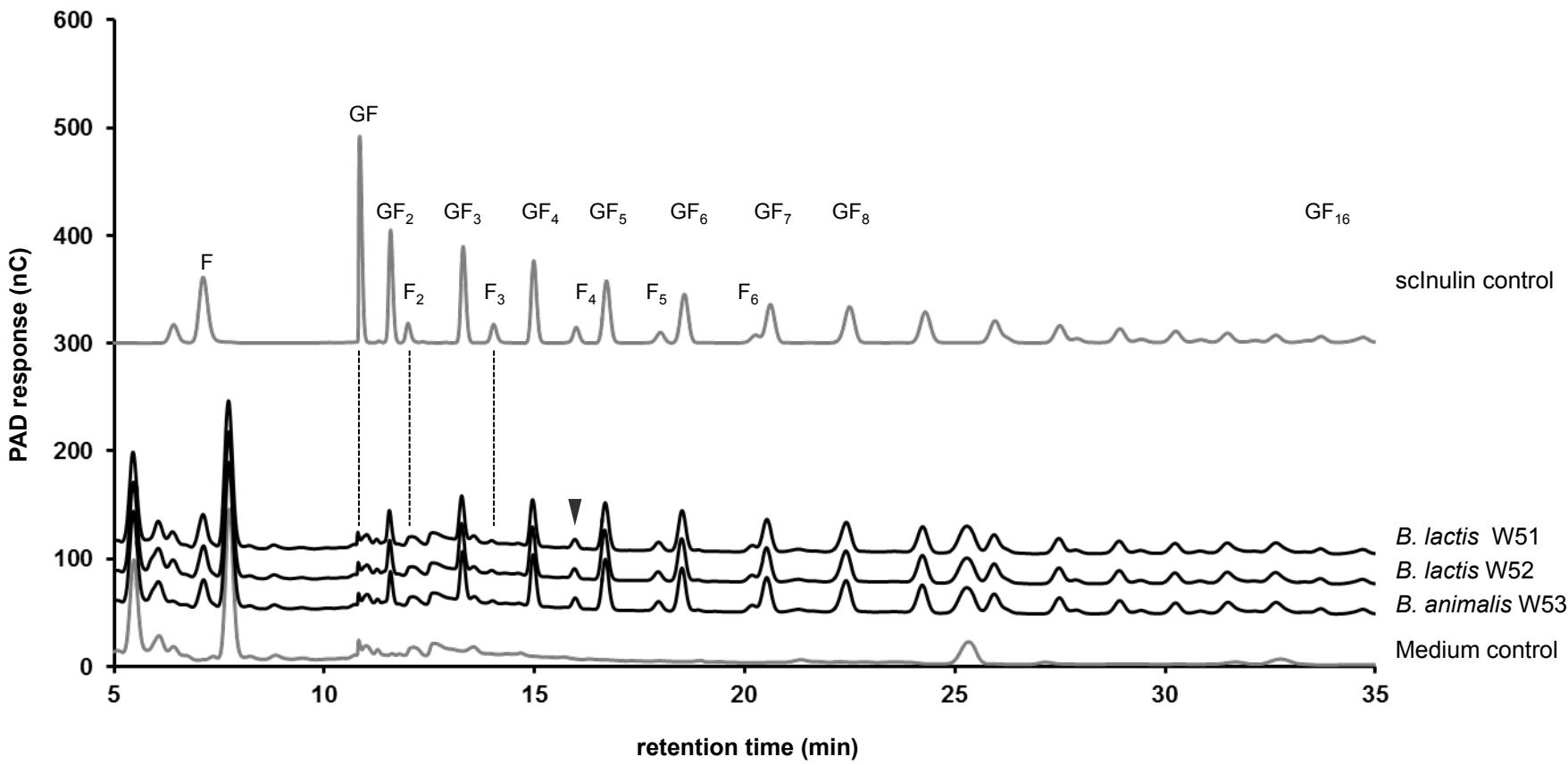


HPAEC-PAD chromatogram of sInulin, components present in supernatants of bacterial cultures grown 18 h

Consumption of (prebiotic) components from scInulin (Frutafit® CLR)

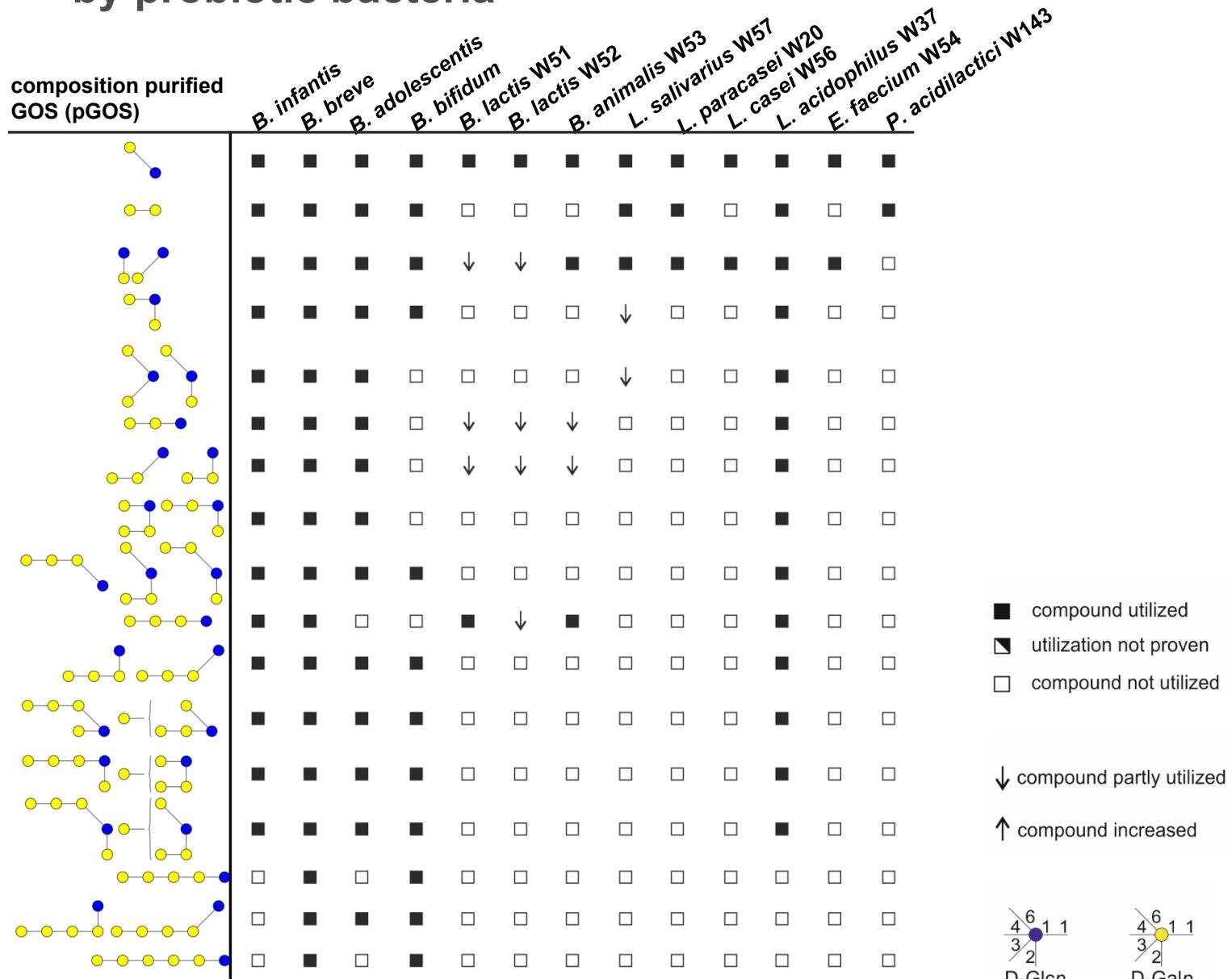


G = D-glucose n = 1 – 40 in scInulin
F = D-fructose



HPAEC-PAD chromatogram of scInulin, components present in supernatants of bacterial cultures grown 32 h

Identification of structure of pGOS components utilized by probiotic bacteria



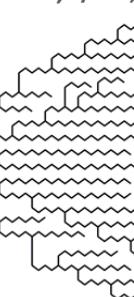
Bifidobacteria grown for 32 h, lactic acid bacteria 18 h

Identification of probiotic strains degrading IMMP



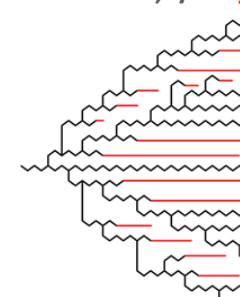
CARBOHYDRATE
COMPETENCE CEN

α -1,4/ α -1,6



GtfB
enzyme

α -1,4/ α -1,6



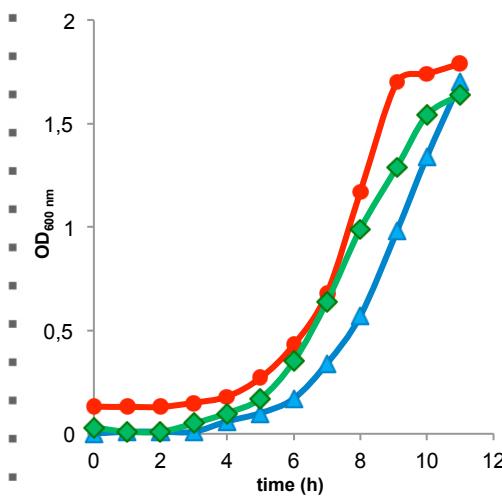
α 1-4

IMMP¹
IMMP²
IMMP³

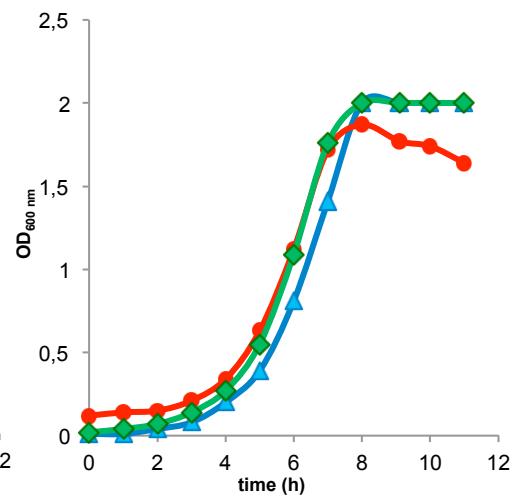
IMMP⁴

α 1-6

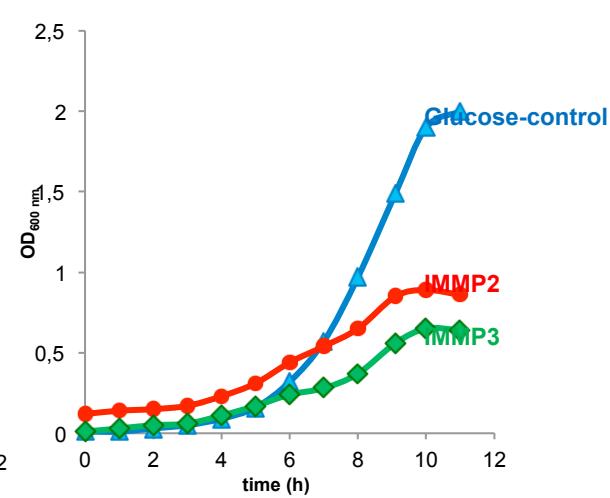
B. breve



B. dentium

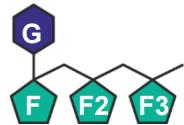


B. adolescentis

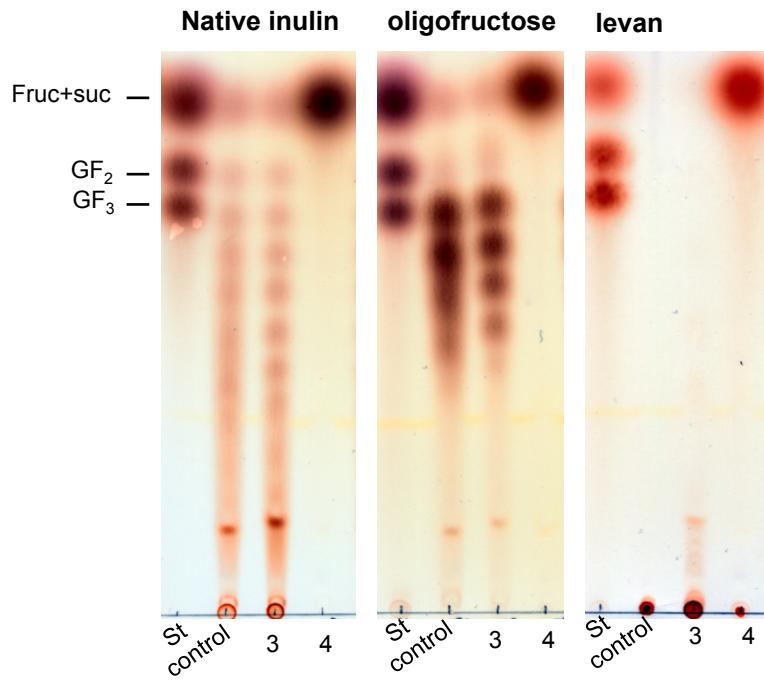
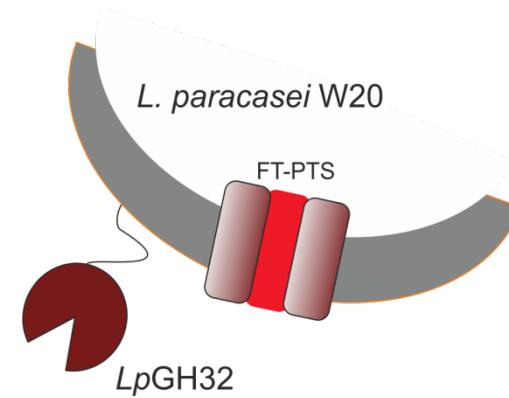


Growth only due to monosaccharides left in IMMPs

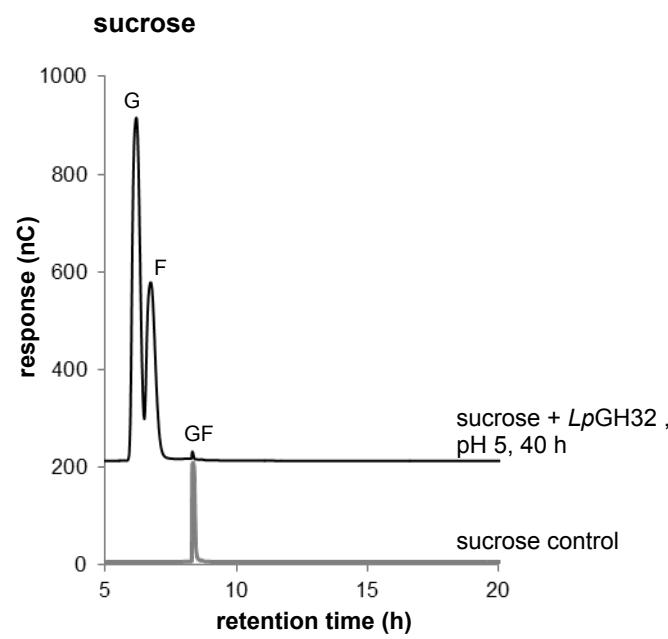
Metabolism of inulin by *L. paracasei* W20



G = D-glucose
F = D-fructose

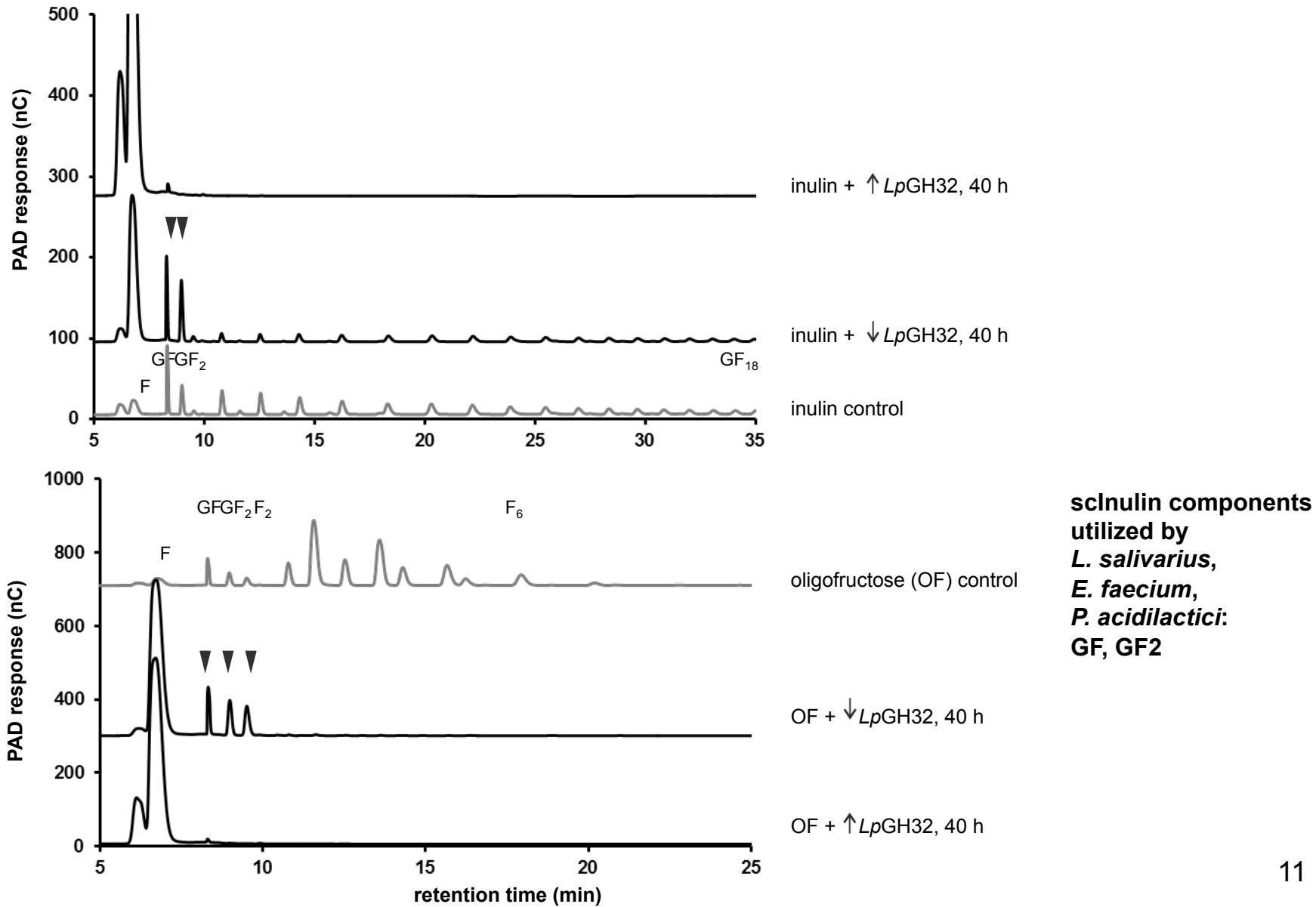


TLC analysis of inulin(-related) substrates by *LpGH32*



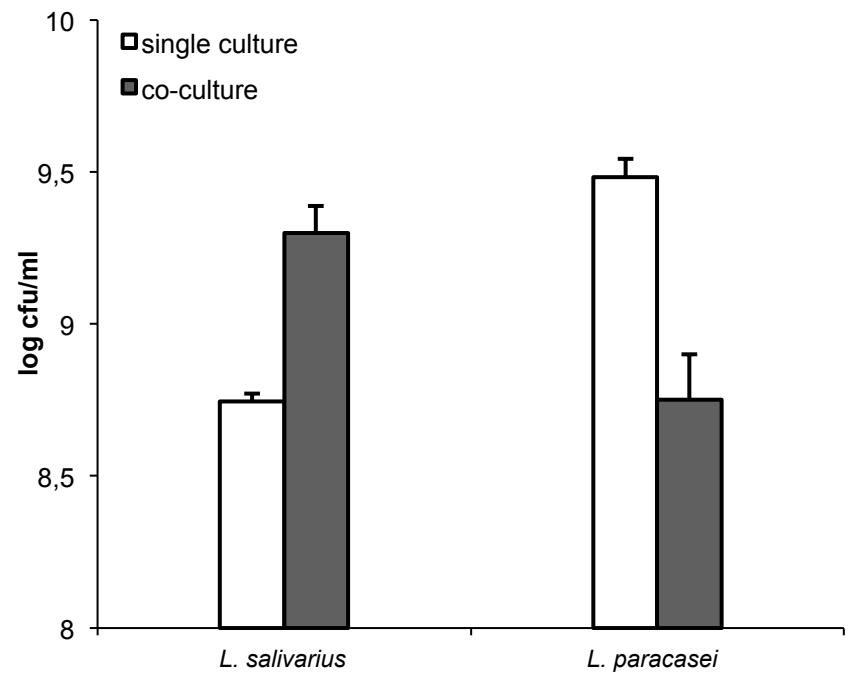
HPAEC-PAD analysis of sucrose converted by *LpGH32*

Time-dependent analysis of *LpGH32* conversion

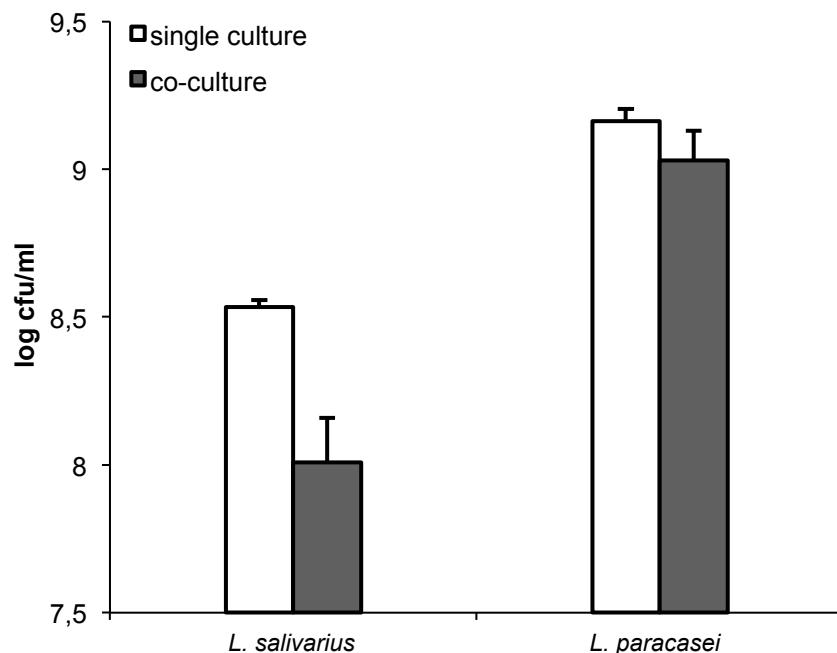


Co-culture experiments *L. salivarius* W57 + *L. paracasei* W20

5 mg/ml scInulin (Frutafit® CLR)

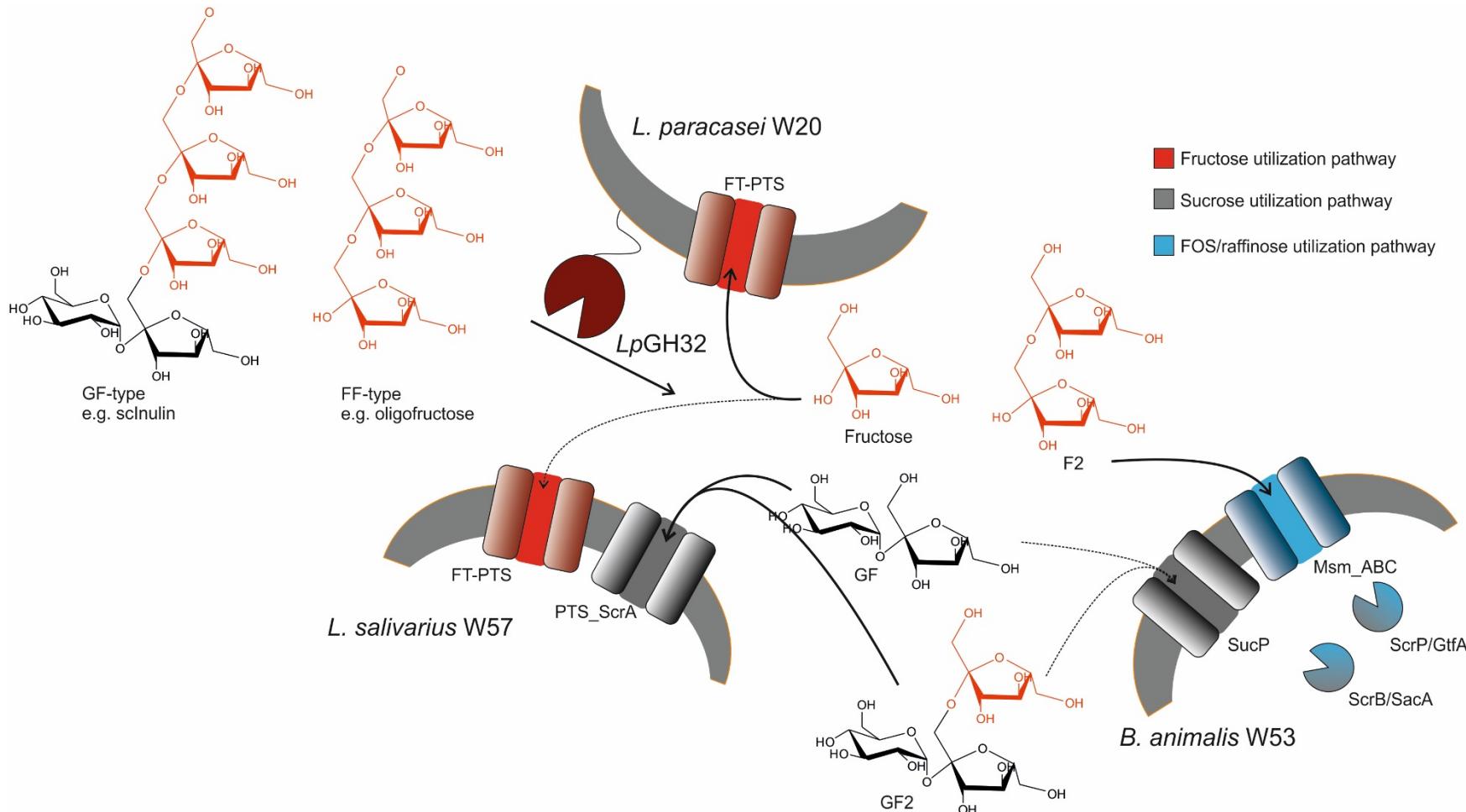


5 mg/ml oligofructose (Frutalose® OFP)



Log cfu/ml obtained from colony counts on MRS Agar plates incubated with diluted samples of single or co-culture (1% (v/v) of *L. salivarius* + 1% (v/v) of *L. paracasei*)

Genome annotation + enzyme characterization



Metabolism of prebiotic $\beta(2\text{-}1)$ fructans by probiotic lactic acid bacteria and Bifidobacteria. Fructose uptake via a Fructose-PTS transport system (FT-PTS); Sucrose uptake via a Sucrose-PTS system (PTS_ScrA), or a Sucrose permease (SucP); FOS(raffinose) utilization pathway with a FOS-ABC transport system (Msm_ABC) followed by intracellular β -fructosidases ScrP/GtfA and ScrB/SacA.

Cross-feeding properties of $\beta(2\text{-}1)$ fructans

Commercial name	Supplier		GF-type (DP)	FF-type (DP)	
Frutalose OFP	Sensus BV	oligofructose	2-7	2-7	e.g. <i>L. paracasei</i> W20 Exo-inulinase + fructose uptake based
P1 FOS	Winckloe BV	oligofructose	2-7	2-7	
Frutafit TEX	Sensus BV	long-chain inulin	10-60		
Frutafit HD	Sensus BV	native inulin	2-60	2-14	
P2 Inulin	Winckloe BV	native inulin	2-60	2-14	
Frutafit CLR	Sensus BV	short-chain inulin	2-40	2-14	
P6 scFOS	Winckloe BV	short-chain FOS	2-6		
Nystose	Carbosynth	GF3	4		
1-Kestose	Carbosynth	GF2	3		



PTS sucrose based uptake
e.g. *L. salivarius* W57

Conclusions



- Probiotic bacteria often utilize specific compounds from (prebiotic) carbohydrates
- Bacteria encode distinct enzymes (e.g. GH32, GH53) and transporters to utilize prebiotic components
- Carbohydrate utilization profiles describe the role of probiotic bacteria in degradation of (prebiotic) carbohydrates
- Determination of metabolic pathways allows formulation of synbiotics based specificities occurring naturally in probiotic bacteria

Acknowledgements



CARBOHYDRATE
COMPETENCE CENTER



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