Extracellular GH32 enzyme of *L. paracasei* W20 enhances the ability of probiotics to utilize a broader degree of polymerization of β(2-1) fructans

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Background

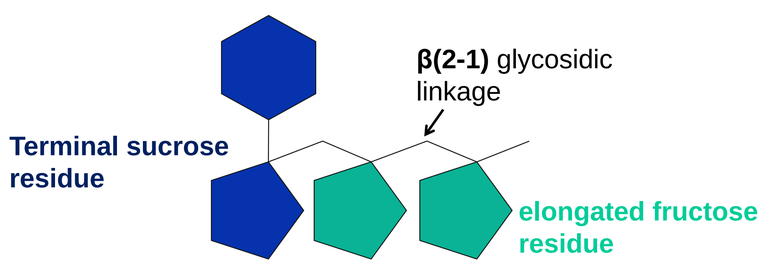
β(2-1) fructans, a group of prebiotic carbohydrates

Unique structural features which determine β(2-1) fructans are

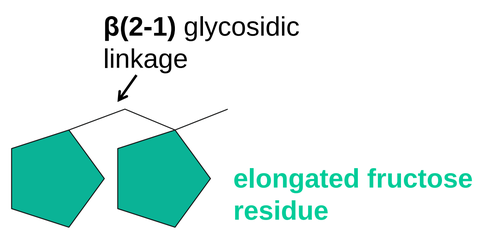
- presence or lack of **terminal sucrose residue** (GF- or FF-type)
- one dominant **glycosidic linkage** (β(2-1))
- variable **degree of polymerization** (DP, 2-60)

Our goal is to study how structural features of β(2-1) fructans influence degradation by probiotic bacteria for development of synbiotics

DP 4 β(2-1) fructan



DP 2 β(2-1) fructan



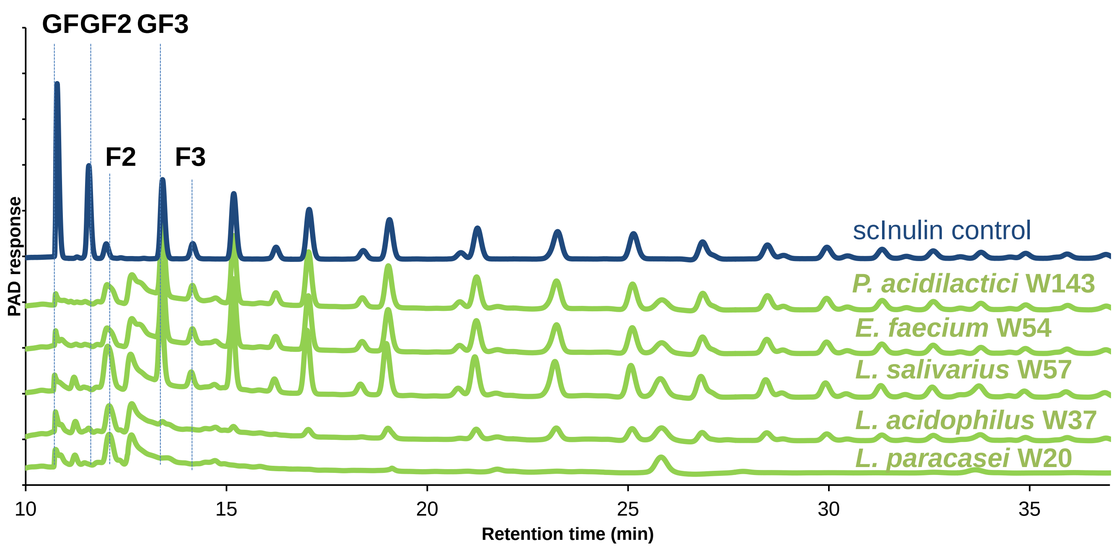
inulin: OF + terminal sucrose, GF-type
FOS: OF + terminal sucrose, GF-type

oligofructose (OF; also FOS named), FF-type

Assessing prebiotic β(2-1) fructan utilization by probiotics with short-chain Inulin (Frutafit® CLR)

%(OD600nm) stationary phase using 5mg/ml scInulin compared to 5 mg/ml glucose

strain	% (OD600nm)
P. acidilactici W143	30.7% ± 0.28
E. faecium W54	45.9% ± 0.43
L. salivarius W57	40.1% ± 0.34
L. acidophilus W37	76.9% ± 1.74
L. paracasei W20	100.4% ± 5.30



HPAEC-PAD chromatograms of scInulin after 18 hr of growth of probiotic strains

Single cultures of probiotic bacteria grew to varied extent on 5 mg/ml scInulin

Probiotic bacteria utilized specific fructan compounds

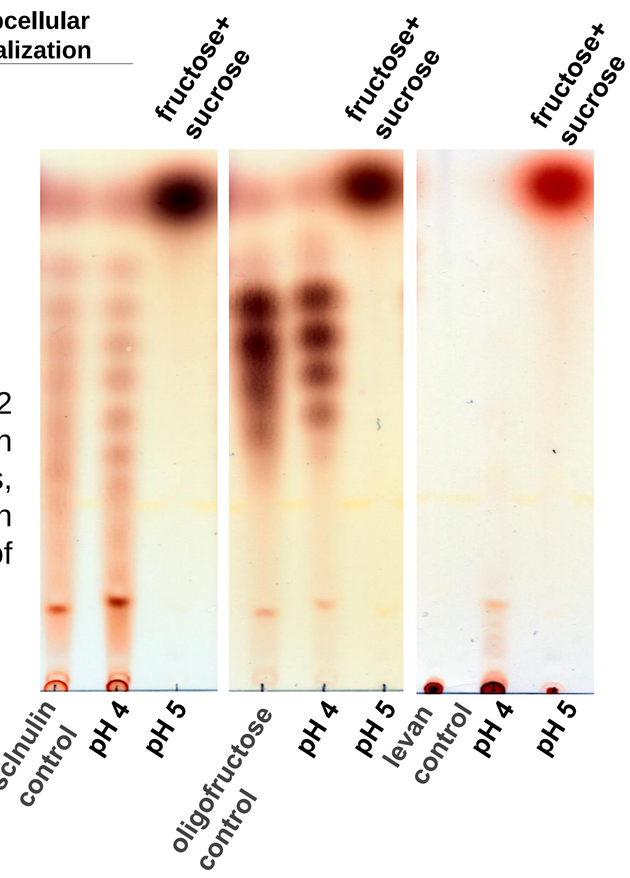
- *P. acidilactici*, *E. faecium*, *L. salivarius*: GF+GF3
- *L. acidophilus*: <GF4, <F4
- *L. paracasei*: all compounds

Characterization of extracellular GH32 enzyme from *L. paracasei* W20

GH32 enzymes annotated in bacterial genomes using dbCAN 5.0
c cytoplasmic, e extracellular

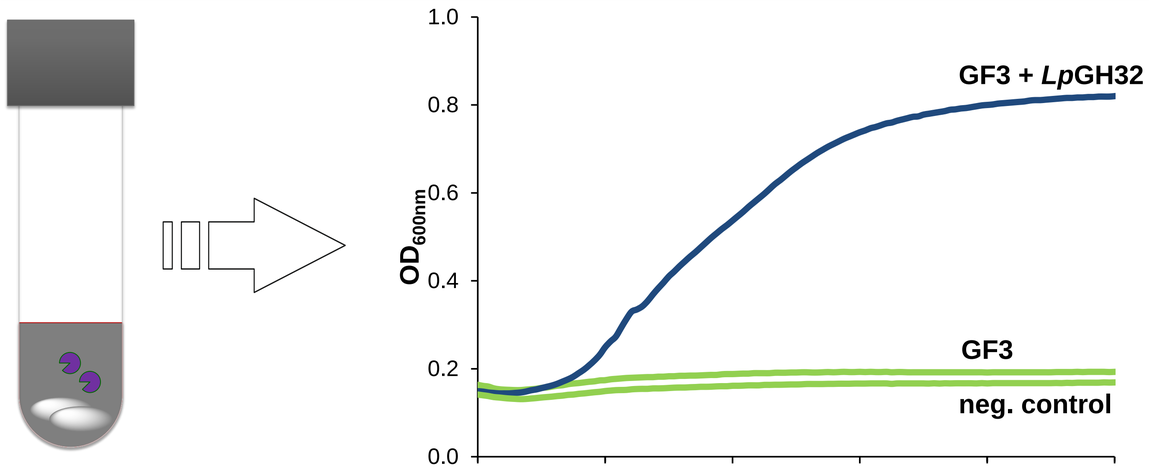
	Number of GH32 enzymes	Predicted subcellular localization
E. faecium W54	1	c
L. salivarius W57	1	c
P. acidilactici W143	-	-
***L. paracasei* W20**	2	c, **e**
L. acidophilus W37	2	c, c

Recombinantly produced *Lp*GH32 enzyme cleaved linkages between fructosyl residues of β(2-1) fructans, sucrose and partly of the β(2-6) fructan levan, thus showing typical activity of an exo-inulinase (EC 3.2.1.80)



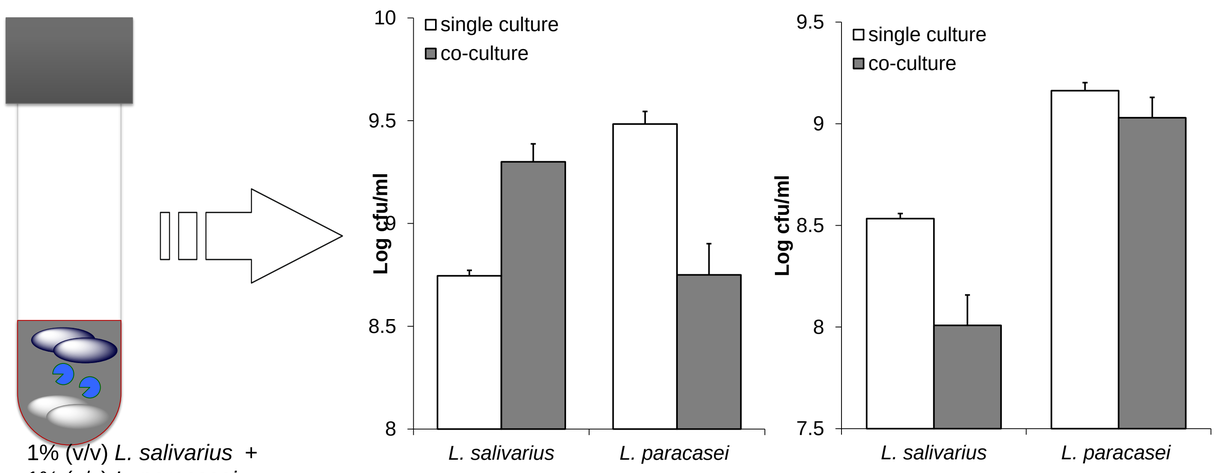
TLC analysis of incubations of recombinant *Lp*GH32 enzyme + 5mg/ml of indicated substrates at pH4/pH5

Growing probiotic *L. salivarius* in the presence of recombinant *Lp*GH32 enzyme



L. salivarius W57 utilizes specifically GF2, but not GF3 (no growth). In the presence of *Lp*GH32 the probiotic utilizes GF3, thus fructan compounds with higher DP

Co-culturing of *L. salivarius* W57 + *L. paracasei* W20 with scInulin/oligofructose



In co-cultures, cross-feeding between *L. salivarius* W57 and *L. paracasei* W20 was observed using scInulin as substrate, but not using oligofructose as substrate

Conclusions

- Probiotic bacteria are highly specific for compounds of β(2-1) fructans
- Specificities can be explained by Carbohydrates Active Enzymes found in bacterial genomes
- Extracellular GH32 enzyme is the key for individual culture of *L. paracasei* W20 to degrade β(2-1) fructans completely
- Addition of recombinant *Lp*GH32 to cultures of other probiotics allows them to utilize β(2-1) fructans with higher DP
- Cross-feeding amongst probiotics occurred on β(2-1) fructans, but to different extent dependent on the substrate structure

Future work

- Explain specificities of probiotics for β(2-1) fructans also at the level of carbohydrate transporters
- Metabolite measurements such as short-chain fatty acids
- Investigation of *in vivo* effects of synbiotic combinations

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