Nutrient Acquisition by Prominent Members of the Human Gut Microbiota

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Introduction

The human gut contains a vast community of microbes known as the microbiota.

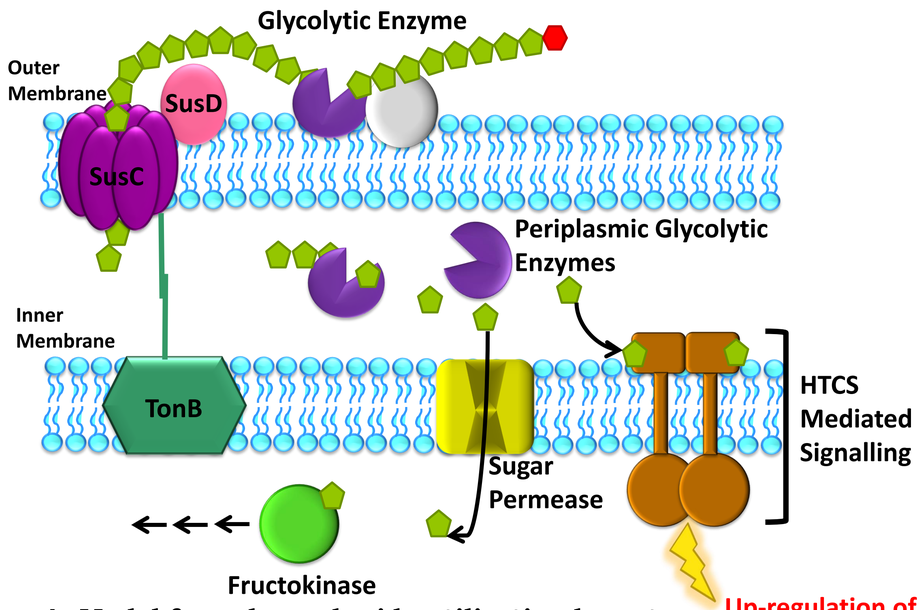
Microbiota colonisation and survival within the gut is dependent on the utilisation of many dietary polysaccharides that are inaccessible to humans.

Degradation of these glycans into smaller more transportable oligosaccharides by Bacteroidetes, one of the two dominant phyla of the gut, requires a complex system of proteins found on the outer membrane and periplasm of the bacteria.

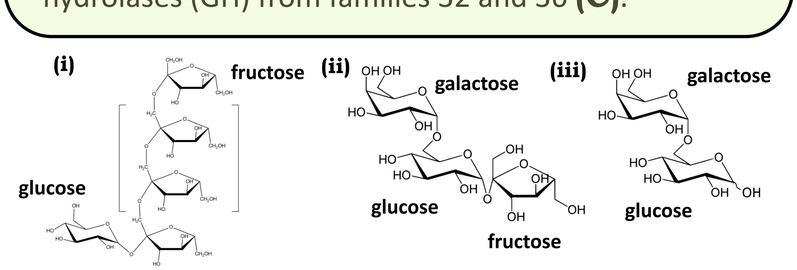
The genes encoding these proteins are found grouped together in clusters termed **Polysaccharide Utilisation Loci (PULs) (A).**

Bacteroides uniformis and B. plebeius activate discrete PULs during growth on fructans, plant derived β-linked fructose polymers that are common components of our diet and often used as prebiotics **(B and C)**.

These PULs encode several putative glycoside hydrolases (GH) from families 32 and 36 **(C)**.



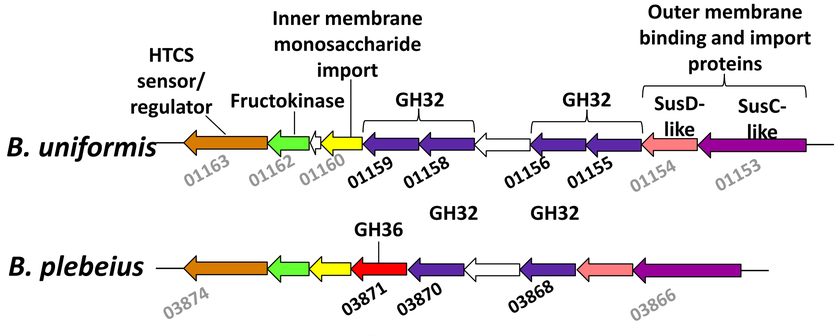
A. Model for polysaccharide utilisation by gut Bacteroidetes



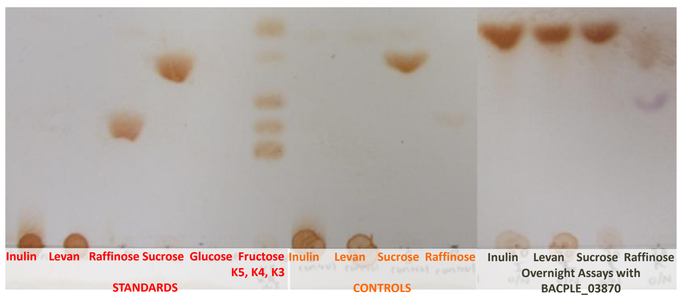
B. Glycan structures (i) Inulin (ii) Raffinose (iii) Melibiose

Aim

To investigate the role of the putative glycoside hydrolase genes from the PULs of *Bacteroides uniformis* and *Bacteroides plebeius* **(C)**.



C. Fructan PULs of *B. uniformis* and *B. plebeius*



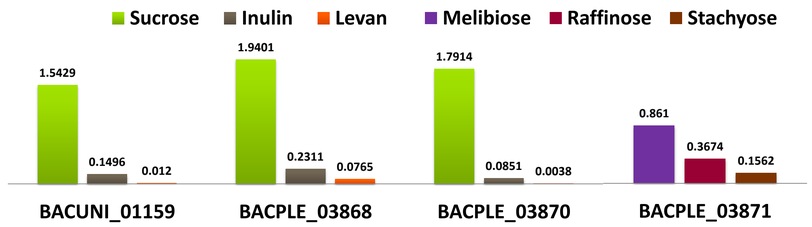
D. TLC analysis of BACPLE_03870 GH32 activity. Release of fructose reveals enzyme has β-fructosidase activity.

Methods

1. Design DNA primers, use PCR to produce multiple copies of the gene.
2. Restriction digest and ligation of gene into expression vector.
3. Transform vector into *E.coli* strain, culture and induce expression of gene.
4. Protein purification by cobalt immobilised affinity chromatography.
5. Enzyme activity assays analysed by thin layer chromatography(TLC) and spectrophotometry.

Results

- Successfully expressed two GH32s from *B. uniformis* (01155 and 01159) and all three target enzymes from *B. plebeius* (03868, 70 and 71)
- TLC shows the cleavage of the terminal fructose from sucrose, inulin, levan and raffinose by the GH32s BACUNI_01159, BACPLE_03868 and BACPLE_03870 **(D)**.
- BACUNI_01155 is potentially an endo-inulinase. Bioinformatic analysis supports this as the most divergent GH32 in the *B.uniformis* PUL and closest homology is to a *B. thetaiotamicron* endo-levanase.
- BACPLE_03871 GH36 is an α-galactosidase. Trial kinetic assays **(E)** show highest activity with melibiose compared to raffinose and stachyose.



E. Reaction rates of the target enzymes versus different substrates

Conclusions

- *B. plebeius* utilises raffinose and stachyose by first removing the fructose with one of its GH32s, followed by degradation of the melibiose product by its GH36.
- *B. uniformis* encodes four GH32s. One of these is likely a surface located endo-inulinase and one a periplasmic β-fructosidase. The role of the other two is not known.

References

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Sonnenburg ED, Zheng HJ, Joglekar P, Higginbottom SK, Firbank SJ, Bolam DN and Sonnenburg JL (2010) *Cell*, **141**: 1241-56.

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