Bacterial Degradation Of Complex Carbohydrates By The Human Gut Microbiota

Romany J McLure
School of Natural and Environmental Sciences
r.j.mclure@ncl.ac.uk

Introduction

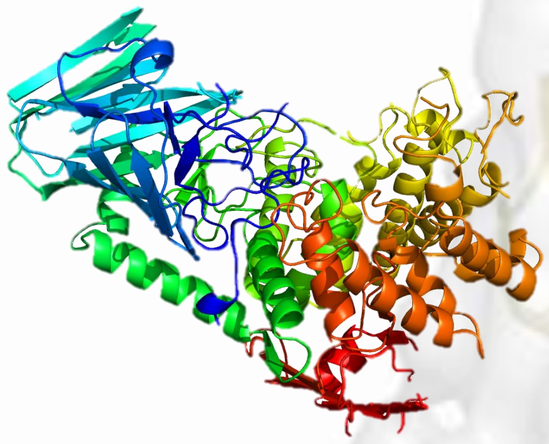

Figure 1. Cartoon structure of BT1032

- The human microbiota aids in metabolism, digestion and immune function of its host
- It is thought that it influences behaviour and neurological functions such as memory and learning (1)
- This project studied *Bacteroides massiliensis*, a gut microbe responsible for the breakdown of complex carbohydrates similar to model organism *Bacteroides thetaiotaomicron*
- N-glycosylation is a post-translational modification whereby a sugar (glycan) is attached to the nitrogen atom of the Asparagine residue of a protein (2)
- Both microbes are involved in the breakdown of High mannose N-glycans (HMNG) that make up a large proportion of the average human diet (3)
- *B. thetaiotaomicron* degrades HMNGs into a Man-α1,6-Man-β1,4 GlcNAc trisaccharide which is degraded by BT1032 (Figure 1)
- This project focused on mannosidase BM00418, the homologue of BT1032, produced by *B. massiliensis*
- The aim was to investigate BM00418 and compare activities and specificities to BM1032 to better understand these enzymes and their functions in carbohydrate utilisation
- Horseradish peroxidase (HRP) was used as a substrate (Figure 2)

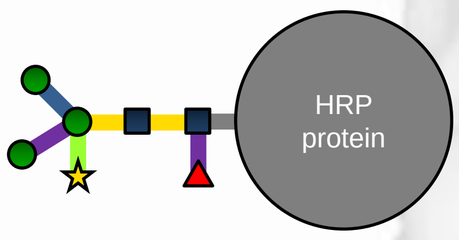

Figure 2. Cartoon structure of HRP

How does *B. massiliensis* degrade HRP?

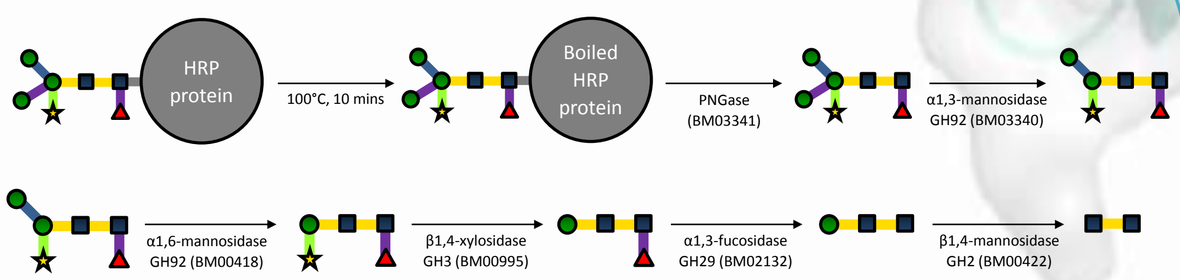

Figure 3. Model of HRP breakdown by *B. massiliensis*

- Different enzymes from *B. massiliensis* were transformed into *E. coli* and the products used to produce different substrates from HRP for BM00418 to be tested on for activity
- BM00418 is an α1,6 mannosidase (GH92)
- BM00418 requires removal of the protein and α1,3 bonded mannose from HRP for activity (Figure 3)

Comparison of BT1032 and BM00418

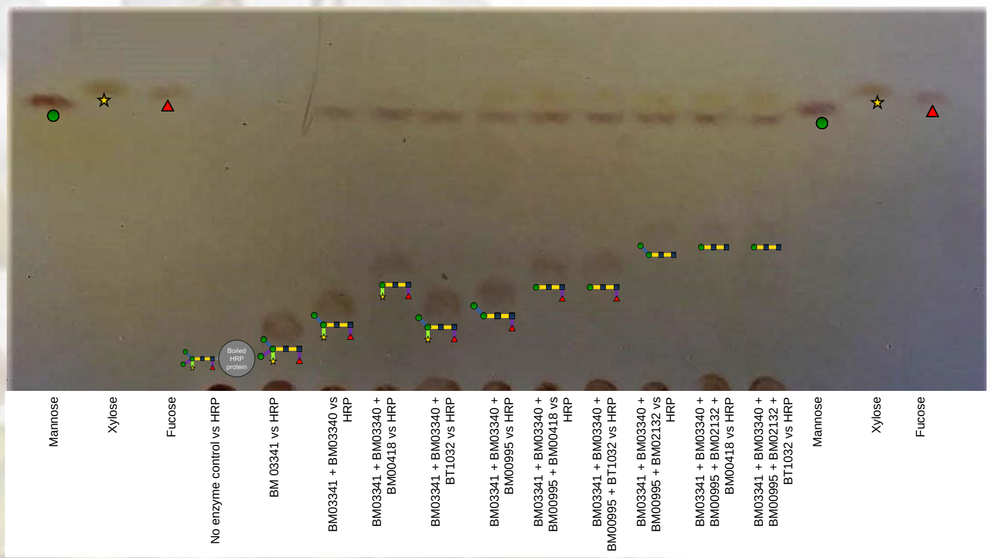

Figure 4. TLC from enzyme assays showing the activity of BM00418 and BT1032 against different substrates

- BM00418 was tested against different substrates and the activity compared to that of BT1032 (Figure 4)
- As with BT1032, BM00418 only shows activity once both the HRP protein and the α1,3 bonded mannose was removed by BM03341 and BM03340, respectively
- Unlike BT1032, BM00418 shows activity on the substrate with xylose
- Both BT1032 and BM00418 show activity on the substrate with fucose removed
- Unlike BT1032, BM00418 does not require GlcNAc at the +2 subsite for activity (3)
- However, Man2A (Figure 5) is preferred and shows higher levels of activity in comparison to single α1,6 mannose bonds (Figure 6)

Figure 5. Cartoon structure of Man2A

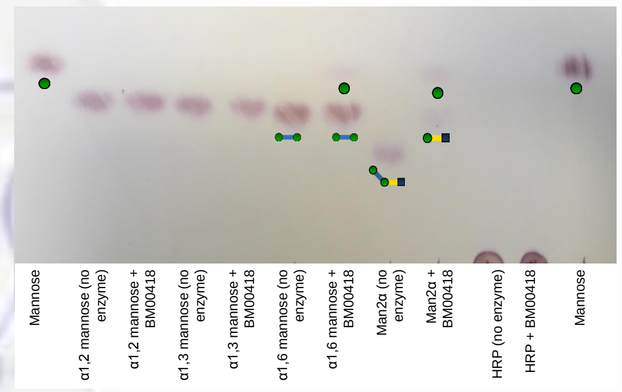

Figure 6. TLC from enzyme assay of BM00418 vs mannose oligosaccharides

Conclusions and implications

- When using HRP as a substrate, both the HRP protein and the α1,3 bonded mannose must be removed for activity of both BM00418 and BT1032. Only BM00418 shows activity when the xylose is attached
- Man2A is the preferred substrate for BM00418 over single α1,6 mannose bonds, but GlcNAc is not required for activity (unlike BT1032)
- This new understanding of the activities and specificities of these enzymes can aid in the development of novel pro- and pre-biotics to encourage a healthy diverse microbiota over an unhealthy microbiota that is often observed in disease states
- Further study would involve mutating specific residues of BM00418 using PCR to determine the role these parts of the amino acid sequence play in enzyme activity and specificity

References

1. Lin C et al. Impact of the gut microbiota, prebiotics, and probiotics on human health and disease. Biomedical Journal.
2. Schwarz F, Aebi M. Mechanisms and principles of N-linked protein glycosylation. Curr Opin Struct Biol.
3. Cuskin F et al. Human gut Bacteroidetes can utilize yeast mannan through a selfish mechanism. Nature.

Acknowledgements - thanks to Fiona Cuskin, Matthew Peake and Lucy Crouch for their support and guidance throughout the project.