Novel enzymes from the human gut microbiota that specifically degrade plant N-glycans

Ekaterina Buzun, Biomedical Sciences BSc, e.buzun@newcastle.ac.uk
Dr Lucy Crouch, Dr David Bolam.

Introduction

N-glycosylation is a post-translational protein modification, the characteristics of which can vary between proteins, species and phyla. Plant N-glycans commonly have an α1,3 fucose decorating the based GlcNAc and a β1,2 xylose decorating the first mannose. (Figure 1)

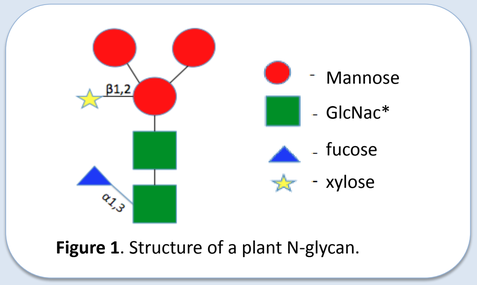


Figure 1. Structure of a plant N-glycan.

A Peptide N-glycosidase (PNGase) from the common gut bacteria, *Bacteroides massiliensis*, was found to target N-glycans with these decorations. The gene for the PNGase (BM03341) is in a polysaccharide utilization loci (PUL) with one other enzyme (BM03340) (Figure 2). This enzyme belongs to the glycoside hydrolase (GH) 92 family, which targets mannosidic linkages. Here we investigated these enzymes on a number of N-glycan substrates. We found that the GH92 is α1,3 specific and has activity when a β1,2 xylose is present, unlike the GH92 from the high mannose N-glycan (HMNG) PUL which was analysed alongside for comparison. We also found that this PNGase is plant-type N-glycan specific. The position of the genes and their complementary activities suggest a plant N-glycan specificity for this PUL.

*GlcNac = N-acetylglucosamine

Aims

- To determine the specificity of BM03340 as well as its activity on mannose-containing N-glycans
- To investigate the specificity of the PUL encoding for BM03340 and BM03341

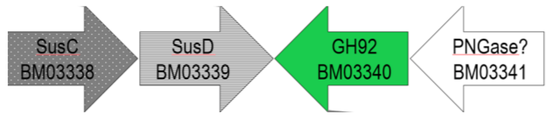


Figure 2. Polysaccharide utilization loci (PUL) encoding for BM03340 (GH92) and BM03341 (PNGase) and their relative positions to each other.

Methods

Enzyme Expression

- The DNA encoding for the enzyme was amplified with the PCR
- DNA inserted into pET21a using Nde1/Xho1 restriction sites.
- TUNER cells were transformed with the appropriate recombinant plasmid and cultured in Luria Bertani (LB) broth to express the protein
- Enzyme was then purified by immobilised metal ion chromatography* and eluted with 10mM and 100mM imidazole.

*The DNA sequence was designed to contain a Histidine-tag on the N-terminus allowing protein purification.

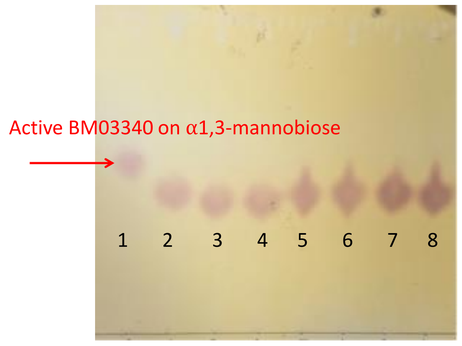
Activity Assays

- Mannosidase activity was detected on the *p*-nitrophenyl α-mannopyranoside (PNP-α-man). A shift from colourless to yellow solution indicated enzyme activity.
- To determine the linkage specificity BM03340 was incubated with α1,2-, α1,3-, α1,4-, α1,5- and α1,6-mannobioses, respectively. Mannose release was observed by thin layer chromatography (TLC) and staining with orcinol.

Specificity assays

- BM03440 alone and in combination with PNGase (BM03341) was incubated with a range of N-glycan substrates and TLC was used to assess activity of the enzymes.
- Another GH92 (BT3991), a published α1,3-mannosidase, was used as a positive control and comparison to the activity of BM03340
- Enzyme kinetics was measured by spectrophotometry using mannose detection kit

Results



1 - α1,3-mannobiose+BM03340
2 - α1,3-mannobiose
3 - α1,6-mannobiose+BM03340
4 - α1,6-mannobiose
5 - α1,4-mannobiose+BM03340
6 - α1,4-mannobiose
7 - α1,2-mannobiose+BM03340
8 - α1,2-mannobiose

Figure 3. TLC showing the activity of BM03340 on α1,3-mannosidic linkage.

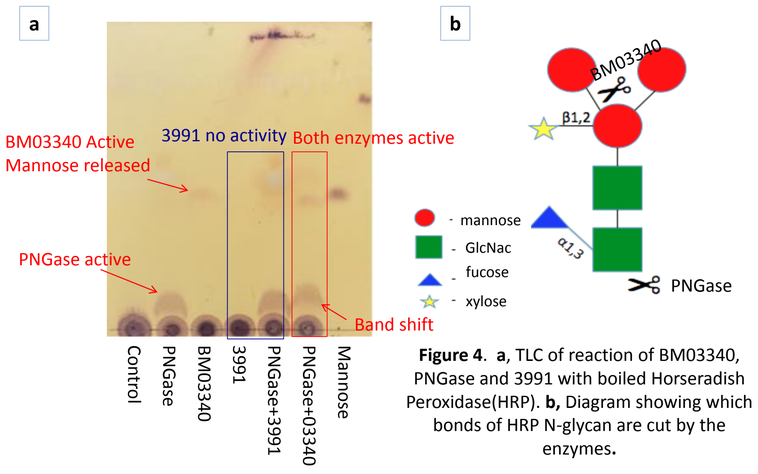
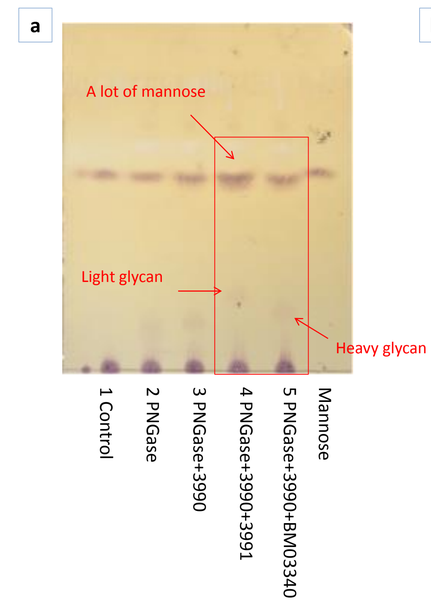


Figure 4. **a,** TLC of reaction of BM03340, PNGase and 3991 with boiled Horseradish Peroxidase(HRP). **b,** Diagram showing which bonds of HRP N-glycan are cut by the enzymes.

Mannose release suggests α1,3-mannosidase activity of BM03340. This is also seen when the enzyme is acting in conjunction with PNGase. Mannose removal makes the N-glycan, which is released by the PNGase, lighter thus creating a band shift. However, the absence of mannose release in reactions where BT3991 is involved indicates the inability of the enzyme to remove α1,3 attached mannose on plant N-glycans.



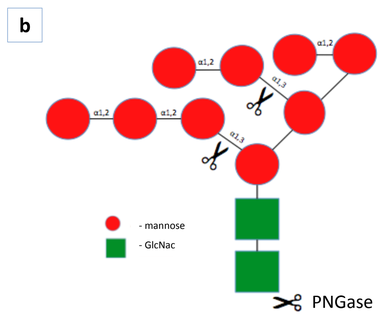
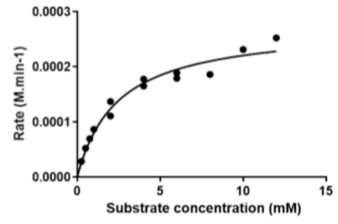


Figure 5. **a**, TLC plate showing activity of BM03340, PNGase and 3991 on RNaseB. **b**, Diagram of the HMNG.

- BT3990 was used to remove α1,2 mannoses from the N-glycan of RNaseB so BM03340 and BT3991 activities could be tested – see comparison between lane 2 and 3.
- The addition of BT3991, in reaction 4, produces a shift in the N-glycan band relative to that seen in reaction 3 and an increased intensity of the mannose band. From the literature, we know that BT3991 removes both α1,3 mannose-linked sugars.
- The addition of BM03340, in reaction 5, also shows a band shift, but one that differs from reactions 3 and 4. This suggests that only one of the α1,3 mannose sugars can be removed by this GH92.



Graph 1. Activity of BM03340 at different concentrations of α1,3-mannobiose. Km=2.420mM ; V_{max}=136.85μM.min^{-1}

Conclusion

- The data suggests that BM03340 is α1,3 specific
- BM03340 removes α1,3 mannose from HRP suggesting plant N-glycan specificity of the enzyme
- BM03340 is able to act in conjunction with PNGase (BM03341) suggesting plant N-glycan specificity of the PUL
- The results however need to be confirmed by performing high performance liquid chromatography (HPLC)
- This research aids our understanding of the role of gut microbiota in the progression or prevention of diseases such as Inflammatory bowl disease (IBD) as levels of protein glycosylation may vary depending on the disease state
- This could also be applied in industry to manufacture new drugs to alleviate the symptoms of inflammatory diseases such as IBD