Characterisation of GH43 in *B.ovatus*

Bolam.D, Rogowski.A, Chaytor.L*, Hussain.W
l.chaytor@ncl.ac.uk

Introduction

Our gut contains a vast community of microbes that play a significant role in maintaining normal health and nutrition. Aberrations in the microbial flora and their interactions with dietary polysaccharides can contribute to serious diseases such as IBD, metabolic syndrome and diabetes. *Bacteroides,* a major phylum of the bacterial flora, are able to utilise many natural polysaccharides in our diet. Their ability is vested from gene clusters known as polysaccharide utilisation loci (PULs). Functions of many of these are yet to be characterised. PULs encode an array of cell envelope associated proteins, tailored to metabolize a specific glycan – called **Sus-like system.**

Aims

•Characterise genes or gene homologues from *Bacteroides* to gain an insight into what type of polysaccharide structure is digested and what is released
•Specifically to characterise a Glycoside hydrolase 43 family in *B.Ovatus*

Results

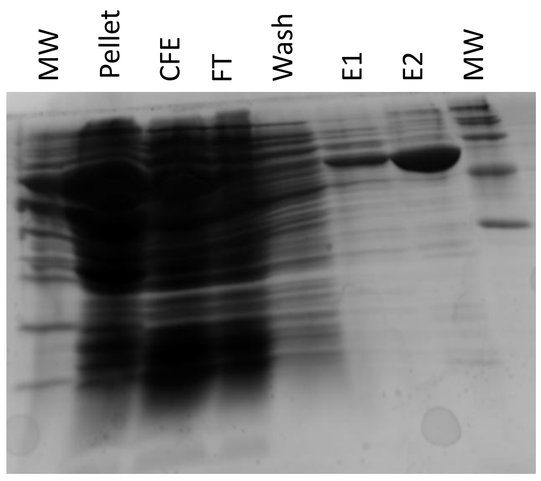


Figure 1.
GH43 was expressed in BL21 E.coli cell strain, and induced with 1mM IPTG.

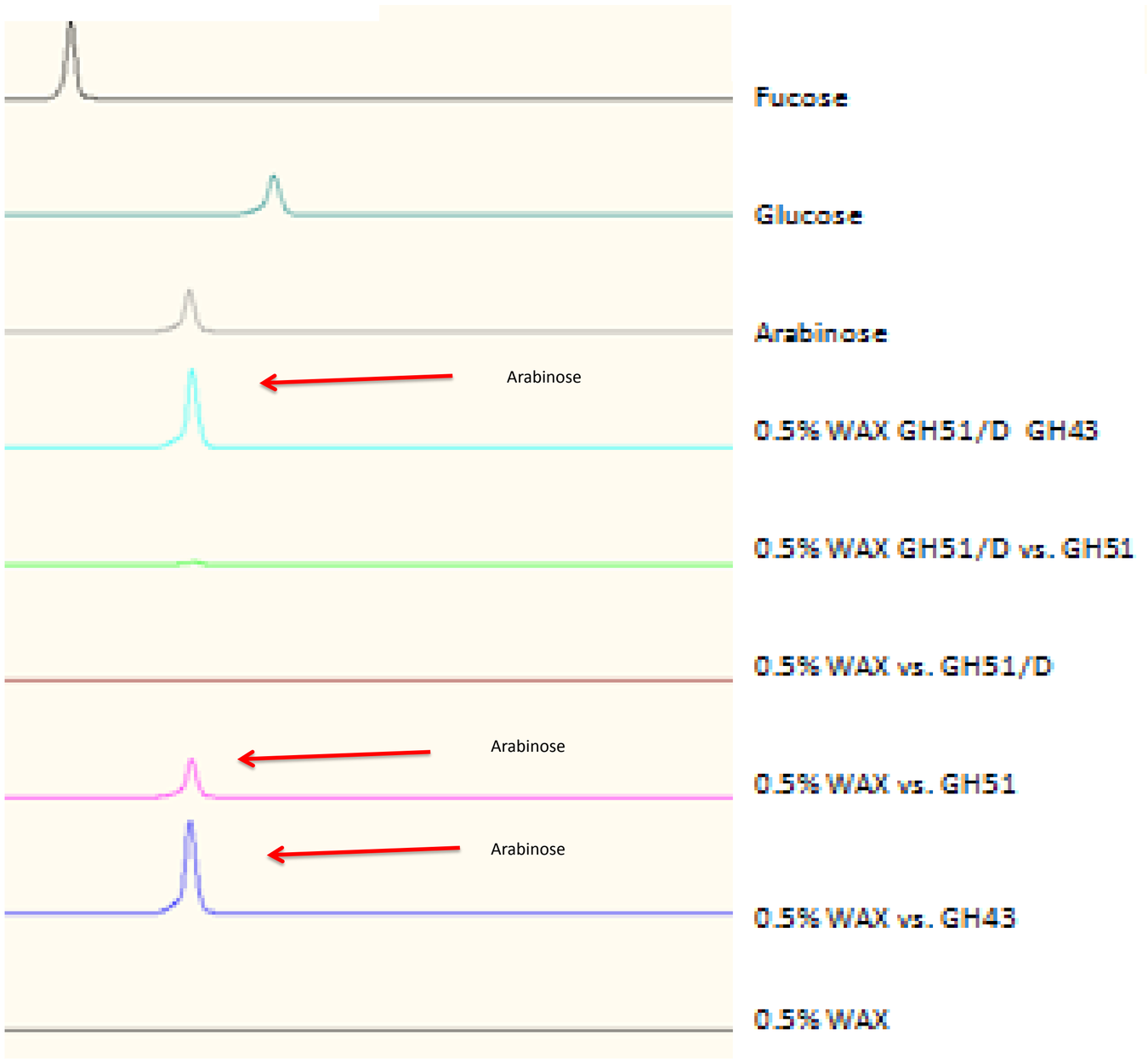


Figure 2. HPLC used to show released production from digestion.

•The HPLC verifies arabinose in released when Wheat arabinoxylan (WAX) is treat with GH43.
•Once the single arabinose side chains have been removed by GH51 the GH43 is added.
•The further release of arabinose from this digestion is the from double arabinose side chains.

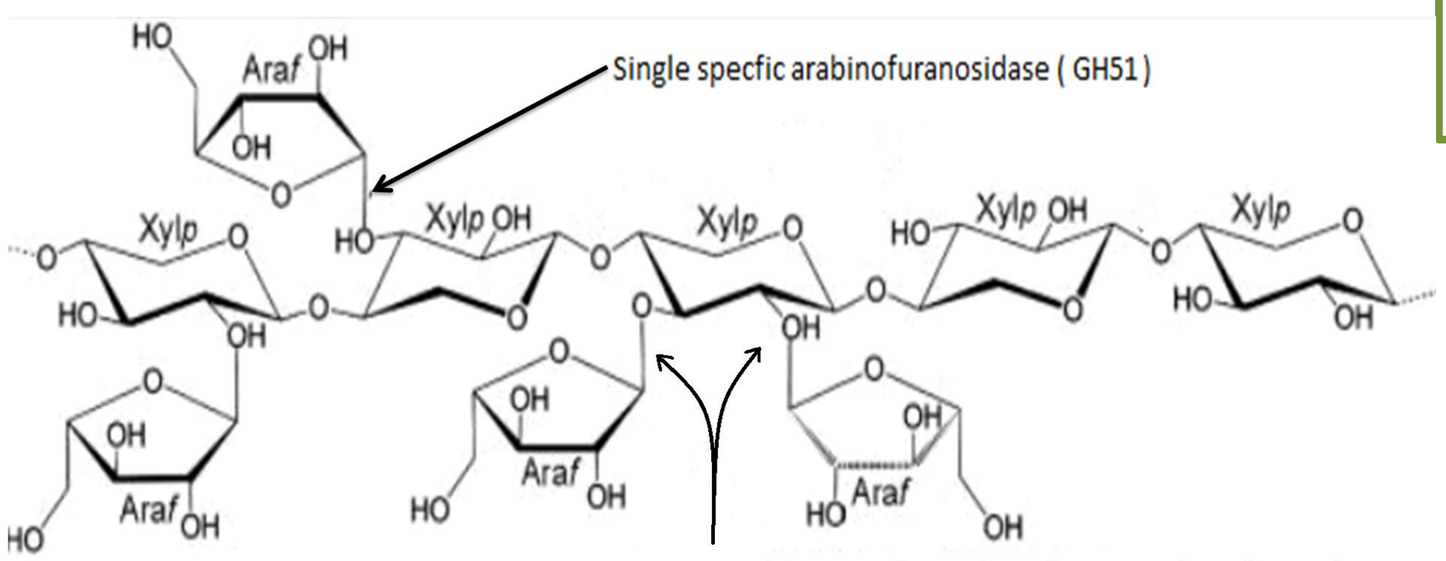
Methods

•GH43 was purified from E.coli
•1µM GH43 was incubated for 2 hours with an array of different xylans (0.5%)
•The reaction was ceased and the reaction mixture was spotted on a TLC plate
•Arabinose was released from arabinoxylans (data no shown)

•GH51(a single specific arabinofuranosidase) was used to remove single arabinose side chains
•Dialysis was used to remove the cleaved single arabinose side chains
•The sample was digested with GH43
•Aliquots from each were ran on HPLC

Conclusions

•That GH43 in *B.ovatus* is a arabinofuranosidase
•Verification that GH43 is a double specific cleaving arabinofuranosidase



Double specific arabinofuranosidase (GH43)