Investigating the kinetics of methyl-lyases in two newly discovered AGP-PULs (5 and 65) in Bacteroides thetaiotaomicron.

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Introduction and Aims

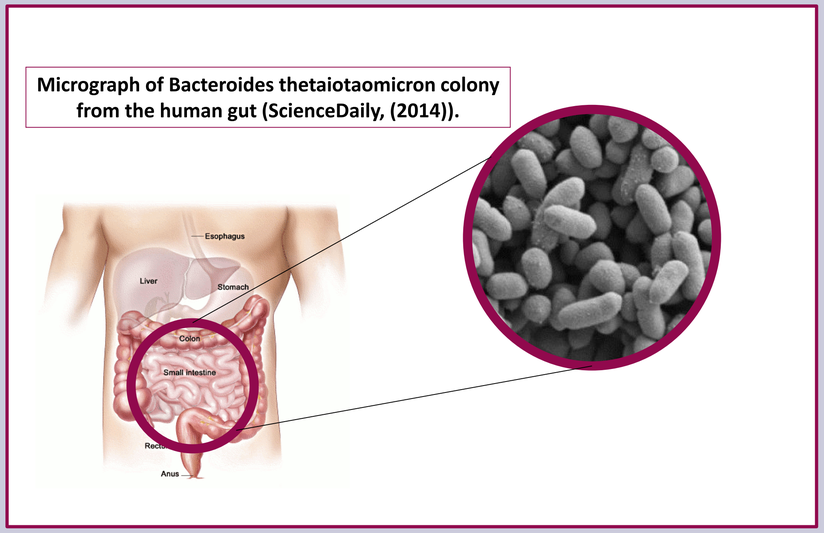
Bacteroides thetaiotaomicron (BT) are just one of the many 'good' bacteria that make up the human gut microbiota. These aid us in the digestion of complex arabinogalactan proteins (AGPs) that we ingest regularly.

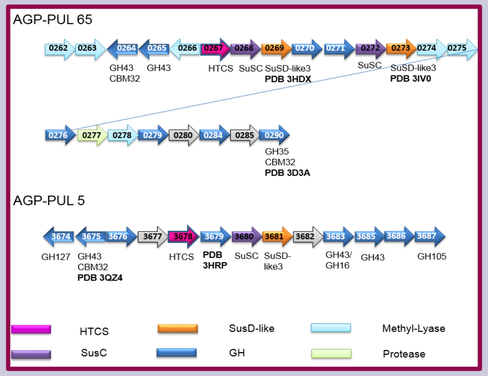
The main sources of AGP are gum arabic, larch wood and wheat although the specific structures of these are not well known. Manufacturers use AGPs as a food stabiliser in products such as M&Ms and marshmallows.

BT have loci called AGP-PULs coding for the expression of enzymes enabling it to break down AGPs. Within the laboratory, two new AGP-PULs were discovered (see diagram to the right). My role involved investigating the kinetics of methyl-lyases (PLs) within these PULs.

Main aims:

- To become more confident in the lab with equipment and techniques
- To express the specific proteins in mutant E.Coli and purify them
- To determine the optimum pH and cofactors each protein requires
- To produce Michaelis-Menton graphs for each PL
- To carry out crystallography screening on each PL





Methods

Firstly, the E.coli was transformed with a mutant plasmid corresponding to a specific PL, grown and induced into expression through the use of IPTG. The proteins were then extracted from the cells and purified using a talon column. Upon isolation of the enzyme, an acrylamide gel electrophoresis was carried out to ensure the correct protein had been obtained.

Numerous protein assays were undertaken in a spectrophotometer to determine the optimum conditions for activity. Factors including pH and divalent metal ions were varied to see which conditions were best.

The loss of methyl group from the AGP can be detected at a wavelength of 235nm (UV).

Through altering only the concentration of substrate in each cuvette during the activity assays, a Michaelis-Menton graph could be created to establish the kinetic parameters of the enzyme.

An alternative source of AGP is Chinese white radish. As the structure of white radish is known, this was purified and tested with PL 0275.

Results and Discussion

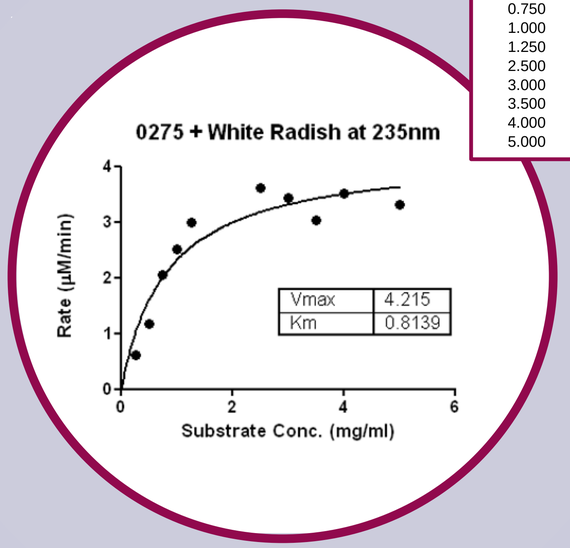
I successfully managed to produce Michaelis-Menton graphs for all of the PLs with gum arabic, each combined with different glycoside hydrolases.

In the last week of my project, I had finally (after much difficulty) obtained white radish AGP. After running activity assays at different pH levels and getting no signal, I assumed that the proteins had become inactive. Upon expressing and purifying new protein, the same thing happened again.

I deduced that the problem must be connected to the enzyme's dependence on something. In previous assays with gum arabic, the reaction required calcium ions but this was not the case with white radish. Instead of calcium, I carried out reactions with several divalent metal ions and found that nickel ions produced a signal.

Unfortunately, after carrying out crystallography screening on each PL I did not obtain any crystals. There was lots of precipitation indicating that I had probably used a concentration of enzyme that was too high. Due to time constraints, I did not repeat these screening methods but focused on the kinetics instead.

Graph to show the rate (µM/min) of 0275 (0.1uM) with different concentrations White Radish AGP + nickel ions



Substrate conc (mg/ml)	Rate (uM/min)
0.250	0.6042
0.500	1.1667
0.750	2.0625
1.000	2.5208
1.250	3.0000
2.500	3.6042
3.000	3.4375
3.500	3.0417
4.000	3.5208
5.000	3.3125

References

1. ScienceDaily, (2014). *How gut bacteria communicate within our bodies, build special relationship*. [online] Available at: http://www.sciencedaily.com/releases/2014/02/140213122358.htm [Accessed 30 Oct. 2014].