How does *Bacteroides thetaiotaomicron* degrade yeast mannan and can it utilise *Candida* mannan as a food source?

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Introduction and Aims

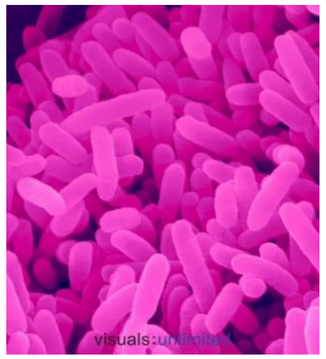

Fig.1: B.thetaiotaomicron cells, x4000 SEM magnification (2)

- Zymolyase is a commercial yeast cell wall degrading enzyme mixture comprising mostly glucanases. **Our aim was to produce a cheap, stable alternative mixture** using enzymes from *Bacteroides thetaiotaomicron,* a dominant gut microbiota member, for use in yeast cell biology.
- *B. thetaiotaomicron* genome contains over 80 Polysaccharide Utilisation Loci (PULs), each orchestrating the degradation of dietary plant and host-derived mucosal glycans (1). PUL treble mutants grow on *Candida albicans* mannan but not the expected *Saccharomyces cerevisiae* α-mannan. **Our second aim is to locate this novel *Candida* glycan utilising enzyme and the gene that encodes it.**

Method

The minimum enzymes required to degrade the yeast cell wall, see (Fig.2), were purified using pET-based recombinant plasmids in *E. coli* strain BL21 (DE3) cells. Thin Liquid Chromatography enzyme assays (TLCs) were performed with a variety of enzyme mixtures acting on *S. cerevisiae*. All TLCs were compared to standards comprising mannose to mannopentose (M1-5).

Secondly, to find this β-1,2-mannase, membrane proteins were purified from Bacteroides triple PUL knockout culture; using 20mM HEPES+ 0.5% sodium sarcosine and 1.5% LDAO + 10mM HEPES + 50mM NaCl detergent washes to collect periplasm (CFE), Inner membrane (sarcosine) and outer membrane (LDAO) fractions.

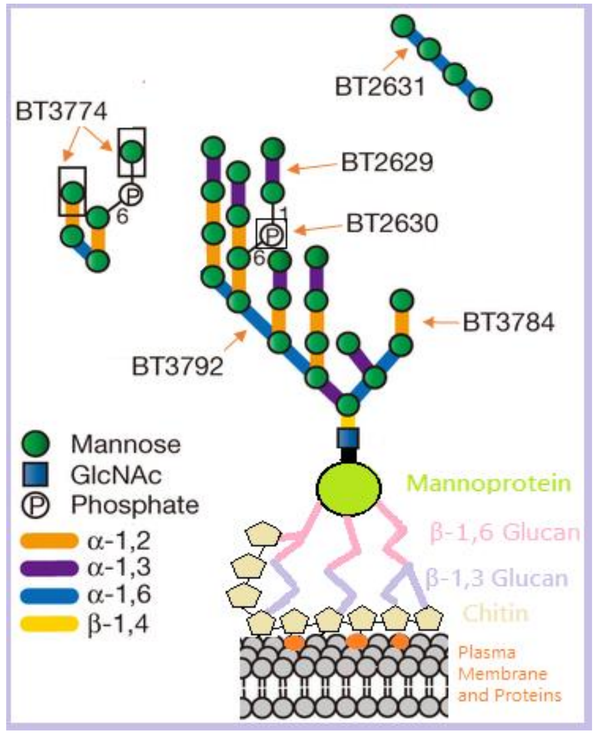

Fig.2: Enzymes shown were chosen based on yeast cell wall linkages and structure. Additionally, BT3312, GH16 and GH81 β-1,3 glucanases (3) (not shown) were purified as well.

Results and Discussion

None of these enzymes substantially degraded *S. cerevisiae* cell wall, individually or in varying mixtures even though these enzymes were shown to be active on β-1,3-laminarin and yeast mannan. Our hypothesis of the importance of mannanases over glucanases is still disputable.

Originally, a TLC of the supernatant of *Candida* revealed oligos that disappear with β-1,2-mannosidase. Therefore an endo-acting β-1,2-mannase must be on the surface of *B.thetaiotaomicron*.

Purification of membrane proteins found mannanase activity in all fractions (Fig. 3) due to multiple enzymes, rather than the believed orphan gene. This indicates a possible PUL of GH76 and GH92 enzymes working on β linkage hydrolysis of *Candida* mannan, not α, due to its inability to grow on *S. cerevisiae* α-mannan. A unique signalling molecule, downregulated by *S. cerevisiae,* could be responsible for regulation of this PUL for starving *B.thetaiotaomicron* to use *Candida* as an alternative food source.

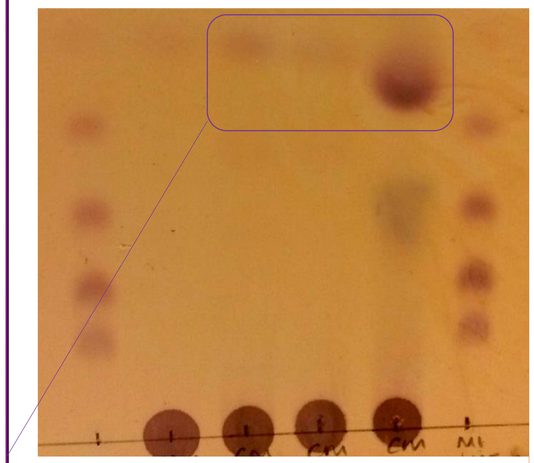

Fig.3: All three fractions were tested against *Candida* mannan. Mannanase activity was observed in all three, due to presence of product oligos, mainly in the CFE/periplasm fraction but light activity also seen in the inner and outer membrane fractions. We would have expected activity in the outer membrane fraction as the target enzyme should be on the outer surface to explain the presence of β-1,2 mannan in *Candida* supernatant culture. This large activity in the other fractions gives rise to the question of multiple enzymes

Conclusion

This project did not generate a viable zymolyase alternative and the mechanism of yeast cell wall degradation is still under debate. We have however **found existence of a possible *Candida* β-mannan degrading PUL** and finding these genes and enzymes will be the next step. Knowledge of carbohydrate utilisation is vital in understanding our microbiota to maximise our health. I wish to thank the Wellcome Trust for funding and to Professor Harry Gilbert and his team for supervising and guiding me in this enjoyable project.

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