EXPLOITING PROKARYOTIC CHITIN-BINDING PROTEINS FOR GLYCAN RECOGNITION

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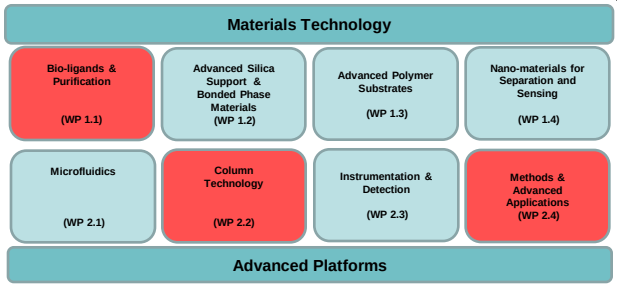
AIMS:

- The cloning, expression and characterisation of prokaryotic chitin-binding proteins from *Serratia marcescens*, *Pseudomonas aeruginosa*, *Photorhabdus luminescens* and *Photorhabdus asymbiotica*
- Development of an assay to assess the activity of chitin-binding proteins
- Mutagenesis of chitin-binding proteins to alter glycan recognition patterns



1. Cloning of Chitin-Binding Proteins

Chitin-binding proteins were amplified from genomic DNA using PCR and subsequently cloned into protein expression vectors.

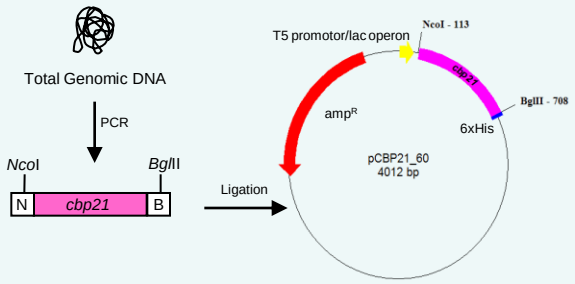


Figure 1: *Overview of the cloning of cbp21.The cbp21 gene was cloned into the pQE60 vector from Qiagen. The MSC is located before the (His)6 amino acid sequence (blue) which allows for the expression of C-terminally (His)6 tagged proteins. This is under control of the T5 promotor/lac operon (yellow). The bla gene encodes beta-lactamase which confers ampicillin resistance to the bacteria (red).*

2. Expression of Chitin-Binding Proteins

Recombinant prokaryotic chitin-binding proteins were expressed in *E. coli* under the control of the lac operon with a C-terminal poly histidine tag, to facilitate downstream purification using Immobilised metal affinity chromatography (IMAC).

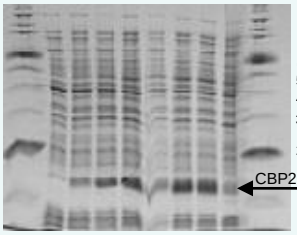


Figure 2: *Time course expression analysis of CBP21 in E. coli KRX. Optimisation of expression of CBP21 resulted in up expression yields of up to 3.1 mg/g of cell paste*

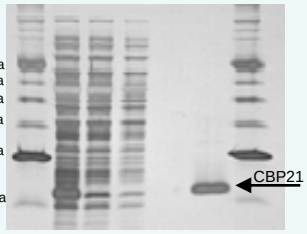


Figure 3: *IMAC purification of CBP21. Analysis of purification fractions resulting from IMAC purification of CBP21 reveals that CBP21 purifies to homogeneity.*

3. Assessing the activity of Chitin-binding proteins

Enzyme linked lectin assay (ELLA) analysis revealed that CBP21 was not capable of interacting with protein attached glycans in its wild type state (Figure 4). A novel assay to assess chitin-binding activity was developed using PAA-linked $(GlcNAc)_N$ polymers (Figure 5).

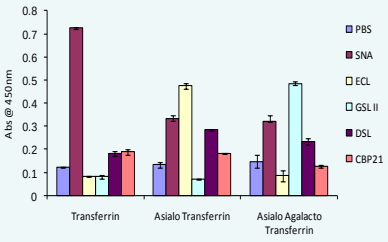


Figure 4: *ELLA analysis of CBP21.*

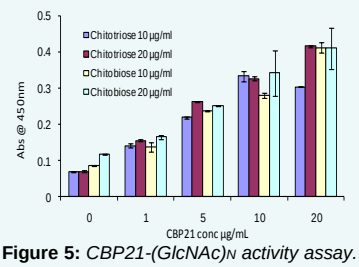


Figure 5: *CBP21-$(GlcNAc)_N$ activity assay.*

4. Mutagenesis of Chitin-binding proteins

Site directed mutagenesis of amino acids thought to be involved in chitin-binding was undertaken (Figure 6). Mutagenesis did not improve the affinity for protein attached glycans, although some differences in affinities for the insoluble substrates; chitin, chitosan and cellulose were observed (Table 1).

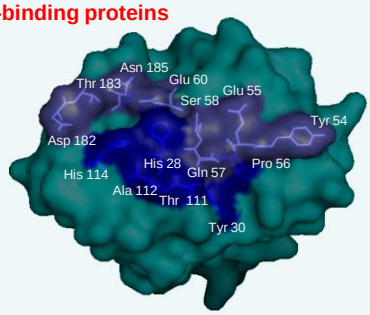


Figure 6: *Residues involved in CBP21 binding to chitin.*

Table 1: *Overview of changes in affinities of CBP21 mutants.*

Protein	β-chitin	α-chitin	Chitosan	Cellulose
WT	√	√	√	√
Y54A	--	--	√	X
E55A	--	√	√	√
P56A	-	-	√	√
Q57A	--	-	++	√
S58A	--	-	√	√
E60A	-	-	√	--
T111A	--	√	+	++
H114A	X	--	+	-
D182A	--	√	√	++

√ Binding comparable to WT, slight increase in affinity compared to WT (+), larger increase in affinity compared to WT (+ +), slightly decrease in affinity compared to WT (-), larger decrease in affinity compared to WT (- -), no binding (X).

5. Project Outputs

Poster Presentations

- **ISSC Review Meeting,** DCU, June 2010.
- **DCU School of Biotechnology Research Day,** DCU, January 2010.
- **UNCSR 10th Anniversary Symposium,** The Helix, Dublin City University, October 2009.
- **9th Jenner Medicine and Glycobiology Conference,** Royal Academy of Medicine of Belgium, Brussels, September 2009.

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