Aims:

• The cloning, expression, purification and characterisation of recombinant prokaryotic glycolytic enzymes
• The mutagenesis of prokaryotic glycolytic enzymes to generate novel recombinant carbohydrate binding proteins
• The characterisation of the binding profile of the novel recombinant carbohydrate binding proteins

1. Intro:
The overall objective of this project is to generate novel carbohydrate binding proteins for use in glycoprotein analysis which are amenable to large scale production. The approach used here is the modification of prokaryotic glycolytic enzymes. The enzymes being studied are from Photobacterium luminescens and are chitobiases (chb) which are a subset of glycolytic enzymes. These enzymes recognize and cleave N-Acetylglucosamine (GlcNAc) dimers to GlcNAc monomers.

We will eliminate their enzymatic activity while hoping they still retain their binding capabilities. The resulting novel carbohydrate binding proteins will have huge commercial potential in the field of glycoanalysis.

2. Cloning, Expression and Purification of a chb from P. luminescens

a) Construct map. Chb PCR product (yellow) was inserted into a pQE60 vector as an Ncol-BamHI fragment. This vector adds a C terminal His tag (red) to its product. The vector also contains ampicillin resistance (blue) for selection. This product was subsequently transformed into Escherichia coli XL10Gold for expression. b) Samples from E. coli cultures containing the expression vectors were taken every hour for 5 hours and lysed by sonication. SDS-PAGE was used to examine expression of chb c) IMAC purification of chb

3. Enzymatic activity of the chb chitobiase compared with commercial counterpart


5. ELLA analysis detects binding of chb mutant to GlcNAc residues

6. Future Work

• Enable detection of chb mutant in ELLAs – use of linker, longer His tag or biotinylation
• Further characterisation of binding once detection is enabled
• Random mutagenesis to alter binding specificities/affinities

7. Project outputs

Oral Presentations
• School of Biotechnology Seminar Series, DCU, April 2011

Poster presentations
• School of Biotechnology Research Day, DCU, January 2011
• ISSC Review Meeting, DCU, June 2010
• 3rd Annual Meeting of Glycoscience Ireland, UCD, August 2010.

This research has been funded by Science Foundation Ireland under grant number and the IRCSET Post Graduate Scholarship Scheme