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Cloning, expression and characterisation of a novel chondroitin AC lyase from *Bacteroides thetaiotaomicron.*

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Background

Within the gut microbiome are billions of bacteria with enzymes (CAZymes) specialised to breakdown specific carbohydrates that the host cannot metabolise and derive nutrients from[1]. These CAZymes are of significant interest, but many have only been predicted based on sequence data. ***Bacteroides thetaiotaomicron***, a strain within the gut microbiome, has two gene sequences predicted to code for Chondroitin AC lyase[2], an enzyme that has significant biomedical applications, including the synthesis of Low Molecular Weight Chondroitin Sulfate (LMWCS) for the treatment of inflammatory disorders[3].

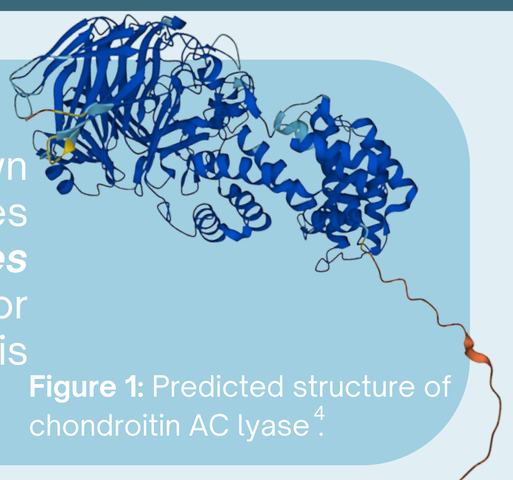

Figure 1: Predicted structure of chondroitin AC lyase[4].

Aims

This project aimed to clone, purifiy and characterise a novel CAZyme, **Chondroitin AC lyase**, based on two predicted sequences from *Bacteroides thetaiotaomicron*.

Methodology

- **Molecular cloning:** Two sequences, Q8A2F5 (CL) and Q8A3J0 (CAC) were isolated, amplified and ligated into one of two plasmids for transformation into *E. coli* cells.
- **Expression:** The *E. coli* cells were induced to allow gene expression. Various conditions were tested to determine the appropriate conditions and plasmid to produce soluble proteins.
- **Purification:** Affinity chromatography was used to purify the enzymes. The HIS-tag was cleaved from the enzymes, and the enzymes were concentrated down and further purified using size exclusion chromatography.
- **Analysis:** Thin layer chromatography was used to determine enzyme activity and substrate.

Results

Protein cloning and expression:

Following cloning, ligation and transformation into *E. coli* cells, the cells were induced under different conditions to determine the conditions and plasmid that produced the most soluble protein.

The black arrows in the SDS-PAGE results highlight the column with the greatest amount of soluble protein. Lanes 1dE and 3dE (figure 2) had the highest amount, so further enzyme expression was induced using the HISTEV plasmid at 15°C, overnight.

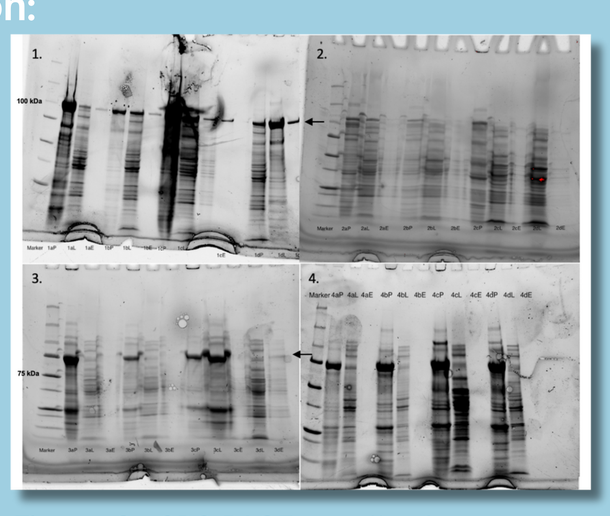

Figure 2: *SDS-PAGE results for enzyme solublility following the induction of E. coli cells using IPTG. 1. CL + HISTEV, 2. CL + 10HIS, 3. CAC + HISTEV, 4. CAC + 10HIS. Induction conditions: a. 37°C, 3 - 4 hours. b. 25°C, 3 - 4 hours. c. 25°C, overnight. d. 15°C, overnight. For each condition, the cell pellet (P), unpurified protein (L) and purified protein (E) were compared.*

Purification:

SDS-PAGE results determined that the HIS tag was cleaved from both enzymes. In figure 3A, a distinct band was seen around 108 kDa in the flow through and wash step, corresponding with the molecular weight for the CL enzyme.

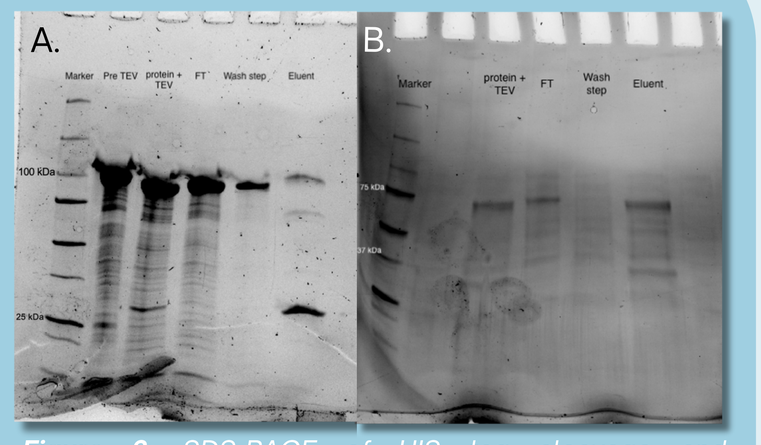

Figure 3: *SDS-PAGE of HIS tag cleavage and purification using affinity chromatography of A. the CL construct, and B. the CAC construct. Samples were taken from each cleavage and purification step for comparison.*

Figure 3B displayed a band around 82 kDa in the flow through step , corresponding with the molecular weight for the CAC enzyme. However, this band was fainter, suggesting that some protein degradation had occurred.

Enzyme activity

Thin layer chromatography (TLC) determined that the CL and CAC enzymes were active. Positive results were seen for both CL and CAC, with the two spots in the reaction conditions showing the chondroitin sulfate had been broken down by active enzymes.

This also confirmed that chondroitin sulfate is a suitable substrate for chondroitin AC lyase.

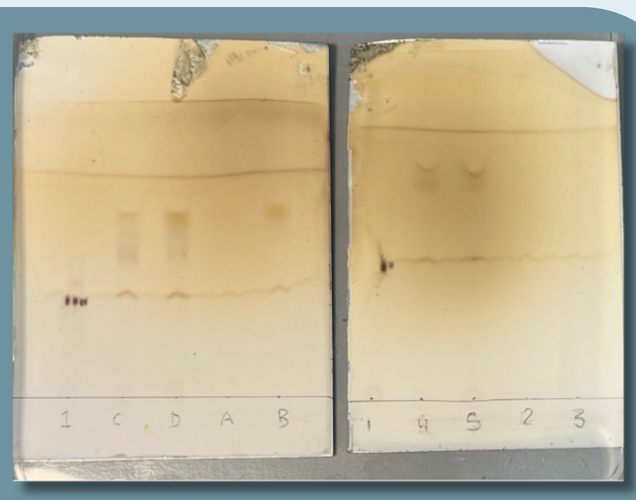

Figure 4*: Thin Layer Chromatography plates for chondroitin sulfate breakdown by CAC (left) and CL (right). 1 . chondroitin sulfate control. C, 4. enzyme and 1 µm chondroitin sulfate. D, 5. enzyme and 10 µm chondroitin sulfate A, 2. 1 µm chondroitin sulfate control, B, 3. 10 µm chondroitin sulfate control.*

Conclusion

This research successfully cloned, expressed and purified two forms of soluble Chondroitin AC lyase from *Bacteroides thetaiotaomicron*, and were shown to have activity with its predicted substrate, chondroitin sulfate. Our research lays the foundations for further research into its biomedical applications, equipping future researchers to explore more characteristics of chondroitin AC lyase.

References

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4. *Jumper, J et al. Highly accurate protein structure prediction with AlphaFold. Nature (2021).*

Acknowledgements

The completion of this research would not have been feasible without the generosity of Lord Laidlaw and Laidlaw Foundation, for the funding, talks and personal development enabled by the scholarship. My greatest thanks are extended to my supervisor, Dr Tracey Gloster, and Verena Oehler for providing me with guidance, patience and resources to undertake this project.