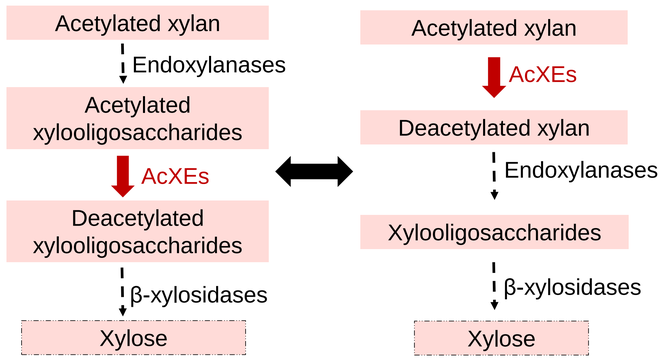
Crystal Structure of a Carbohydrate Esterase 7 Family Enzyme from a Desert Metagenome

Fiyinfoluwa Adesioye[1], Thulani Makhalanyane[1], Surendra Vikram[1], Trevor Sewell[2], Wolf-Dieter Schubert[3] and Don Cowan[1]

[1]Centre for Microbial Ecology and Genomics, Genomics Research Institute, University of Pretoria.
[2]Institute of Infectious Disease and Molecular Medicine, University of Cape Town.
[3]Department of Biochemistry, University of Pretoria.

INTRODUCTION

- Acetyl xylan esterases (AcXEs) hydrolyse acetyl groups that sterically hinder the breakdown of xylans by endoxylanases during bioconversion of lignocellulose.
- Some AcXEs in the carbohydrate esterase (CE) 7 family preferably act on acetylated xylooligosaccharides and/or substrates with ≤C4 acyl groups.
- Novel AcXEs are important for engineering efforts towards improving enzymatic hydrolysis of hemicellulose [1].
- The Namib Desert hypolith metagenome possesses cell wall-degrading enzyme-encoding genes potentially capable of activity under conditions of low water activity and high temperature and alkalinity.



Mechanism of action of acetyl xylan esterases

OBJECTIVES

- To study the structural and mechanistic properties of a novel AcXE of metagenomic origin.
- To identify the structural determinants of thermostability in CE7 AcXEs.
- To investigate the structural properties that drive the substrate specificity of CE7 AcXEs.

MATERIALS AND METHODS

Protein identification, isolation and characterization

- *In-silico* – mining and gene synthesis
- Cloning and expression
- Western blotting and protein purification
- Enzyme activity assays

Crystallization

- Crystallization: Sitting drop
- pH: 8.5
- Temperature: 18°C
- Buffer: 0.1 M Tris HCl / 0.1 M MES
- Precipitant: 20% PEG

Data collection and structure solution

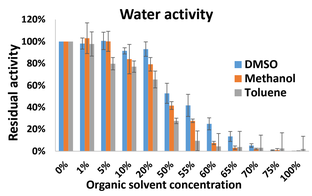
- Data collection: Beamline ID23; ESRF, Grenoble France
- Structure solution: Molecular replacement
- Refinement: Phenix refine
- Validation: Phenix validate and Molprobity

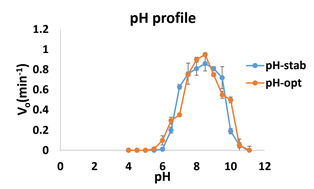
RESULTS

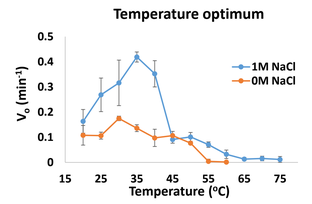
Enzyme Kinetics

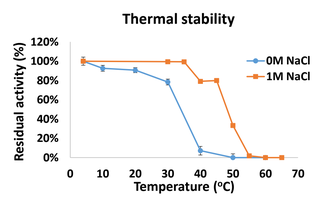
Substrate	p-NPA	4-MUA	2-NA	7-ACA	P-NPB
k_{cat}/K_M ($M^{-1}s^{-1}$)	3.26 x 10^6	3.03 x 10^6	7.84 x 10^5	2.6 x 10^5	1.1 x 10^4

- pH optimum is 8.5 and temperature optimum 35°C in 1 M NaCl.
- NaM1 cleaves artificial esterase substrates such as acetates of p-nitrophenol (p-NP), 4-methylumbelliferyl (4-MUA), 2-naphthol (2-NA), p-NP butyrate, 7-aminocephalosporanic acid and acetylated xylan, with lowest activity for the latter.

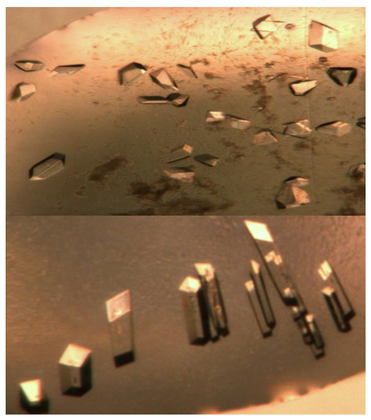








Structural characterisation

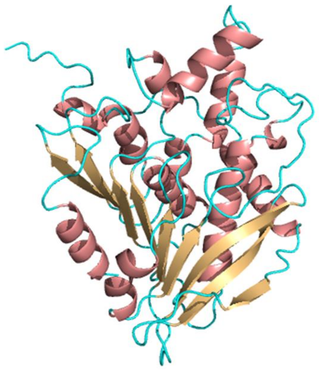


Data collection

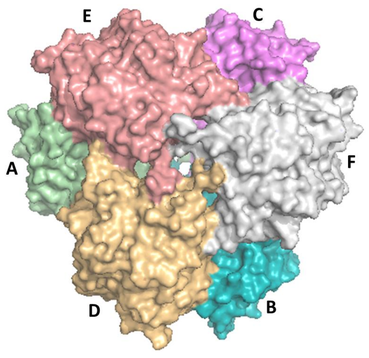
Space Group:	$P2_12_12_1$
Resolution:	89.32 – 2.03 Å
Completeness:	99.9%
Redundancy:	2.0

Refinement

R-free:	21.8%
R-work:	16.6%
RMSD (angles):	0.89 Å
Average B-factor:	20.9 Å^2

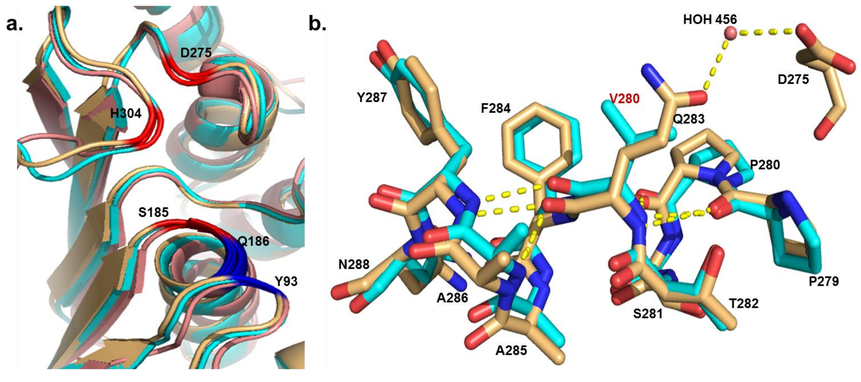


NaM1 tertiary structure



NaM1 quaternary structure

Thermal stability

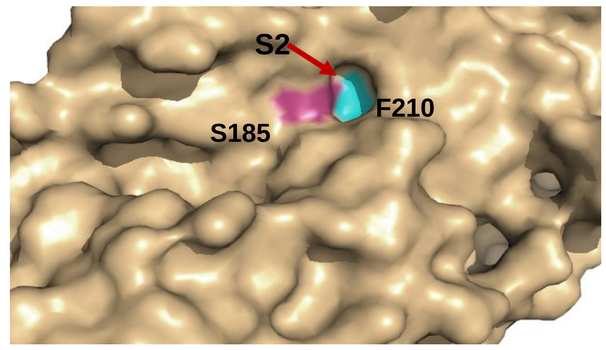


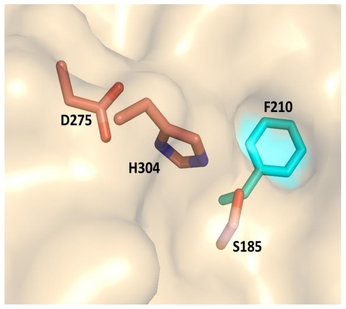
a. Superposition of NaM1 (light orange) with thermostable CE7 enzymes from *Thermoanaerobacterium* sp. (PDB: 3FCY, cyan) and *Thermotoga maritima* (PDB: 3M81, salmon) showing active site residues (red) and oxyanion hole residues (blue).

b. A hydrophobic valine in the strictly conserved PPST**V**FAAYN motif of thermostable CE7 enzymes (cyan) is replaced by a polar Gln283 in NaM1 (light orange) resulting in a water-mediated hydrogen bond to catalytic Asp275.

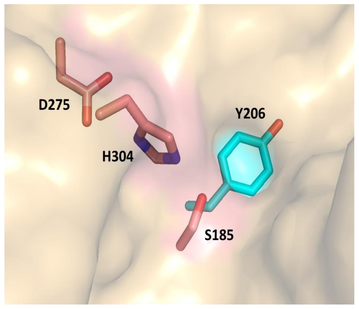
Substrate specificity

The residues at the base [2] and around [3] the S2 binding site determine the length of the acyl moiety of substrates to be catalysed by CE7 enzymes.





NaM1 (This study)



BpAXE (PDB: 2XLB) [2]

CONCLUSIONS AND HYPOTHESIS

- First metagenome-derived CE crystal structure in the PDB.
- NaM1 is a doughnut shaped, homo-hexameric, halophilic and mesophilic AcXE with broad substrate specificity.
- Several structural elements, including a Val→Glu replacement in a strictly hydrophobic region, contribute to make NaM1 the most thermolabile CE7 AcXE analysed.
- Phe210 allows binding of substrates with ≤C4 acyl moieties as opposed to Tyrosine in the same position.

REFERENCES

[1] Biely, Biotechnol. Adv. **2012**, 30, 1575-1588
[2] Montoro-Garcia *et al.*, Biochem. J. **2011**, 436, 321–330
[3] Singh and Manoj, Biochem. Biophys. Res. Comm. **2016**, 476, 63-68

ACKNOWLEDGEMENTS

GRI Genomics Research Institute University of Pretoria
CMEG Centre for Microbial Ecology and Genomics
science & technology Department: Science and Technology REPUBLIC OF SOUTH AFRICA
OWSD