CHALMERS UNIVERSITY OF TECHNOLOGY

INDUSTRIAL BIOTECHNOLOGY

BIOCHEMICAL & STRUCTURAL CHARACTERIZATION OF BACTERIAL CARBOHYDRATE ESTERASE 15 (CE15) MEMBERS

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BACKGROUND

Glucuronoyl esterases (GEs) are a relatively new class of enzymes which cleave an ester linkage connecting lignin to glucuronoxylan (Figure 1A). Putative GEs have been identified in many biomass degrading microbes and are now classified in the Carbohydrate Esterase 15 (CE15) family. Phylogenetic analysis of CE15 members indicates that the family has a wide degree of sequence diversity (Figure 1B). Previously, few GEs have been biochemically characterized and only three protein structures have been determined.

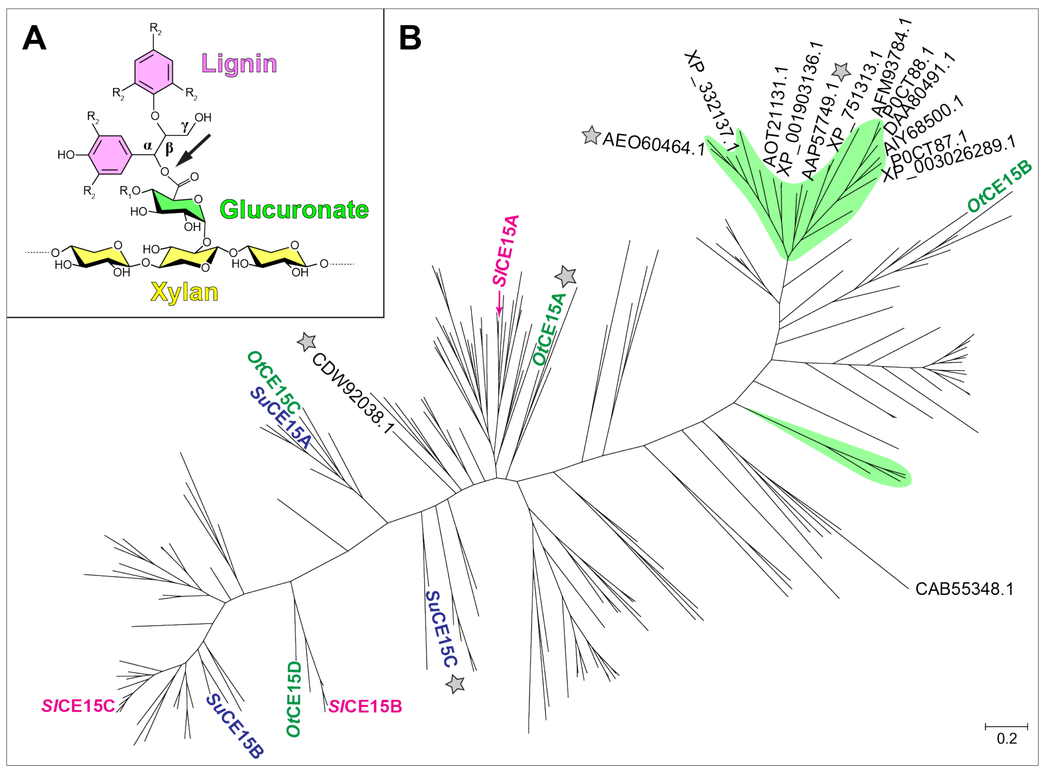
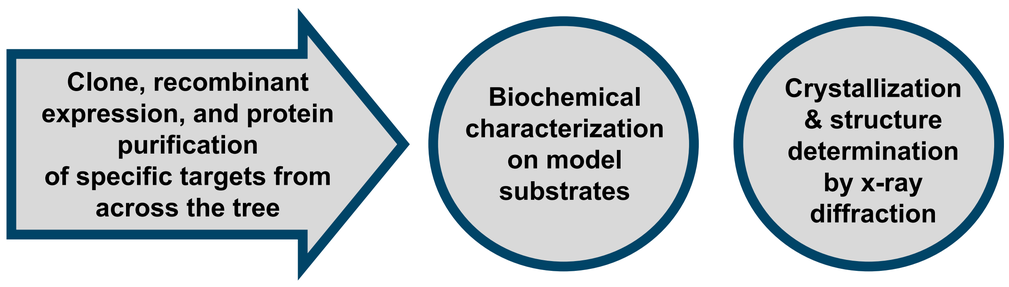


Figure 1: (A) General structure of LCC esters (either α- or γ- linked to glucuronic acid moieties on xylan), and site of enzymatic cleavage by glucuronoyl esterases (arrow). R1 may be either H or a methyl moiety, while R2 labels represent possible further connections to the lignin network. (B) Phylogenetic tree of all CE15 catalytic domains in CAZy. Biochemically characterized members are labelled with their respective Genbank accession numbers. Branches representing members of fungal origin are shaded in green. Stars indicate structurally determined members. Enzymes characterized in this study are labelled with their protein names, color coded in green for O. terrae, magenta for S. linguale, and blue for S. usitatus.

OBJECTIVE

Advance understanding of the CE15 family by biochemically characterizing and determining structures of bacterial CE15 proteins from across the protein family.

METHODS



KINETIC CHARACTERIZATION

While all of the CE15 enzymes have preference for benzyl glucuronoate, some have promiscuous specificity. The pH optimum is dependent on host.

CE15	pH Optimum	k_{cat}/K_m ($s^{-1}M^{-1}$)		
		Benzyl Glucuronate	Methyl Glucuronate	Methyl Galacturonate
*Ot*CE15-A	8.0 – 8.5	4.64×10^3	6.85×10^3	4.85×10^3
*Ot*CE15-B	8.0 – 8.5	1.86×10^1	1.14	8.68
*Ot*CE15-C	8.0 – 8.5	1.16×10^4	8.98×10^2	1.19×10^3
*Ot*CE15-D	6.0 – 8.5	1.11×10^4	5.19×10^2	1.95×10^{-6}
*Sl*CE15-A	6.0 – 6.5	1.88×10^3	1.55×10^3	3.82×10^1
*Sl*CE15-B	6.0 – 6.5	2.60×10^3	4.57×10^2	3.66×10^{-7}
*Sl*CE15-C	6.0 – 7.0	9.69×10^2	1.03×10^2	3.73×10^{-6}
*Su*CE15-A	8.0 – 8.5	2.20×10^4	2.32×10^3	1.62×10^3
*Su*CE15-B	7.0 – 8.0	1.49×10^3	6.00×10^2	9.00×10^{-3}
*Su*CE15-C	7.0 – 8.0	2.27×10^4	1.66×10^4	1.59×10^3

STRUCTURAL INVESTIGATION

We have solved the structure of 2 bacterial CE15 enzymes. The proteins have an insertion, relative to fungal CE15 enzymes, that docking simulations suggest may act as lignin binding pocket.

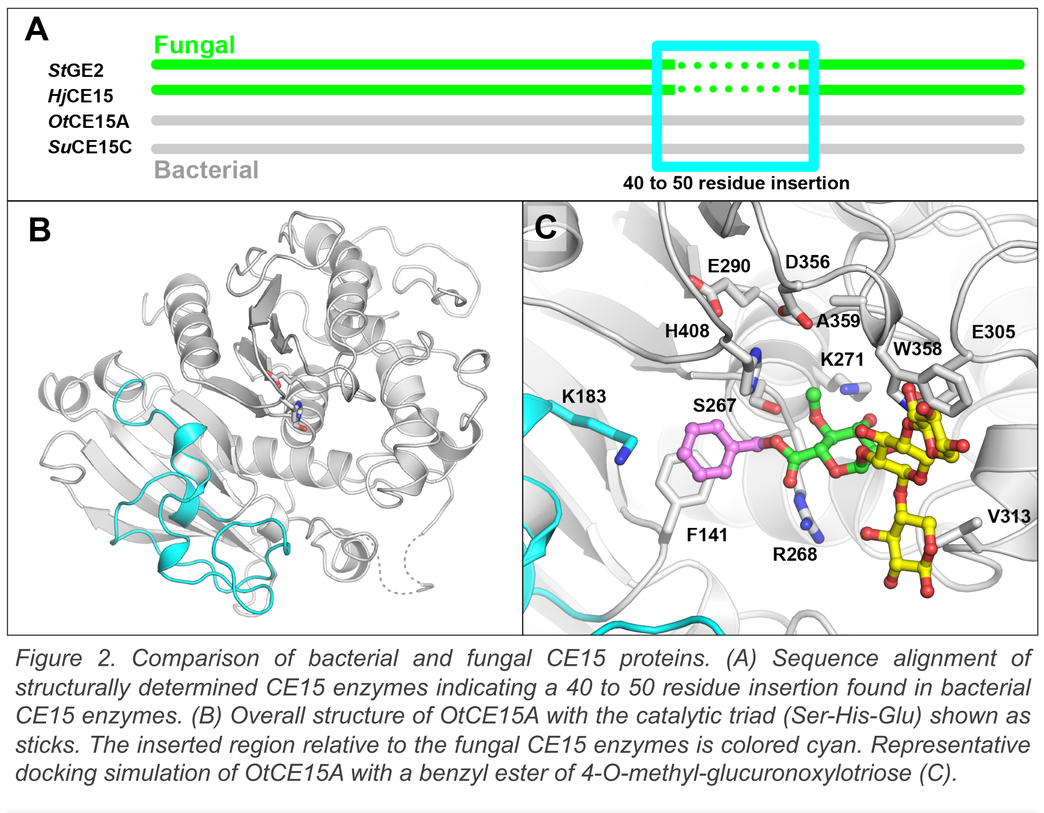


Figure 2. Comparison of bacterial and fungal CE15 proteins. (A) Sequence alignment of structurally determined CE15 enzymes indicating a 40 to 50 residue insertion found in bacterial CE15 enzymes. (B) Overall structure of OtCE15A with the catalytic triad (Ser-His-Glu) shown as sticks. The inserted region relative to the fungal CE15 enzymes is colored cyan. Representative docking simulation of OtCE15A with a benzyl ester of 4-O-methyl-glucuronoxylotriose (C).

FUTURE DIRECTIONS

The results produced have been published [1] and future studies will focus on understanding of specificity and discrimination by co-crystal structures.

[1] Arnling Bååth, J., and Mazurkewich, S., *et al*. (2018) Biotechnol. Biofuels. **11**: 213

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