Family GH157 endo-β-1,3(4)-glucanases exhibit exo-hydrolytic activity

Catarina CASEIRO [1,2], Catarina CASEIRO [1,2], Nicholas MCGREGOR [3], Márcia CORREIA [4,5], Ana Luísa CARVALHO [4,5], Gideon DAVIES [3], Carlos MGA FONTES [6], Pedro BULE [1,2]

[1] CIISA—Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, Univ. of Lisbon, Portugal, [2] Associate Lab. for Animal and Veterinary Sciences, Lisbon, Portugal, [3] York Structural Biology Lab., Dep. of Chemistry, The University of York, UK, [4] UCIBIO, Chemistry Dep., NOVA School of Science and Technology, Univ. NOVA de Lisboa, Portugal, [5] Associate Lab. i4HB, Institute for Health and Bioeconomy, NOVA School of Science and Technology, Univ. NOVA de Lisboa, Portugal, [6] NZYTech Genes & Enzymes, Lisbon, Portugal

ccaseiro@fmv.ulisboa.pt

β-glucans are a class of polysaccharides whose hydrolysis is catalysed by β-glucanases. Due to the complex relationship between the molecular structure and functional profile of β-glucans, there is a continuous need for specific β-glucanases that can fully reveal β-glucans' potential applications [1]. Here we report the first biochemical and structural characterization of two β-glucanases from the novel glycoside hydrolase family 157 (GH157) and investigate their molecular basis for substrate hydrolysis.

Genes encoding the GH157s from the human gut bacteria *Bacteroides cellulosilyticus* and psychrophilic bacteria *Labilibaculum antarcticum*were expressed and structurally characterized by X-ray crystallography. Their specificity and activity were analyzed with reducing sugar assays, enzyme kinetics and product analysis by HPAEC-PAD and LC-MS.

Specificity screening revealed that the enzymes show a preference for mixed-linkage glucans. HPAEC-PAD and LC-MS on hydrolysis products revealed that both enzymes display an endo mode of action, capable of cleaving β1-3 and β1-4-linked glucoses, when preceded by a β1-3 linkage. *La*GH157 structure showed a $(\beta/\alpha)_8$ barrel fold and a retaining mechanism of hydrolysis, with two glutamates serving as the catalytic residues.

This study provides the first characterization of GH157 members, identifying them as retaining endo-β-1,3(4)-glucanases, with exo-hydrolytic activity in the case of *La*GH157. This provides insight into β-glucan deconstruction in the human gut and marine biomes, while identifying potential β-glucan catalysts.

Acknowledgements: We acknowledge FCT, through the grants: UIDB/00276/2020 (CIISA); LA/P/0059/2020 (AL4AnimalS); and 2022.07903.PTDC. We also acknowledge ANI through the grant LISBOA-01-0247-FEDER-047033 [GlycoMed] and the Gilead GÉNESE program through the project 17805. Moreover, we thank the Royal Society (Ken Murray Research Professorship to GJD), the BBSRC (grant BB/R001162/1 to GJD) and the European Research Council ERC-2020-SyG-951231 "Carbocentre" to GJD. We also thank Rachael Hallam and Dr. Leonardo Gomez for skilled maintenance and provision of access to HPAEC-PAD instrumentation. C.Caseiro is funded by an individual PhD scholarship from FCT (SFRH/BD/147152/2019).

Bibliographic references:

[1] C. Caseiro, J. N. R. Dias, C. M. G. A. Fontes, P. Bule (2022), Int. J. Mol. Sci. (23), 3156.