A novel D-xylose isomerase: from the gut of a wood feeding beetle for improved conversion in *Saccharomyces cerevisiae*

Paulo César Silva[a*], Javier A. Ceja-Navarro[bc], Flávio Azevedo[a], Ulas Karaoz[d], Eoin L. Brodie[de] and Björn Johansson[a]

[(a)] CBMA - Center of Molecular and Environmental Biology, University of Minho, Campus de Gualtar, Braga, 4710-057, Portugal.

[(b)] Biological Systems and Engineering, Lawrence Berkeley National Laboratory, Berkeley, California, USA.

[(c)] Institute for Biodiversity Science and Sustainability, California Academy of Sciences, San Francisco, California, USA

[(d)] Earth and Environmental Sciences, Lawrence Berkeley National Laboratory, Berkeley, California, USA.

[(e)] Department of Environmental Science, Policy and Management, University of California, Berkeley, California, USA.

[*] Presenter

Carbohydrate rich substrates such as lignocellulosic hydrolysates remain one of the primary sources of potentially renewable fuel and bulk chemicals. The pentose sugar D-xylose is often present in significant amounts along with hexoses. For low value/high volume products, yield is of paramount importance for process economy. *Saccharomyces cerevisiae* can acquire the ability to metabolize D-xylose through expression of heterologous D-xylose isomerase (XI). This enzyme is notoriously difficult to express in *S. cerevisiae* and only fourteen genes have been reported to be active so far. We cloned a new D-xylose isomerase derived from microorganisms in the gut of the wood-feeding beetle *Odontotaenius disjunctus*. Although somewhat homologous to the current gold-standard from *Piromyces* sp. E2, metagenome scaffold gene neighborhoods and metagenome binning identified the gene as of bacterial in origin and the host as a *Parabacteroides* sp. Expression of the new XI enzyme in *S. cerevisiae* resulted in faster aerobic growth on D-xylose than the XI from *Piromyces*. The D-xylose isomerization rate of the yeast expressing this new XI was also 72 % higher. Interestingly, increasing concentrations of xylitol (up to 8 g/L) appeared not to inhibit xylose consumption in both strains. The newly described XI displayed 2.6 times higher specific activity, 37 % higher affinity for D-xylose, and exhibited higher activity over a broader temperature range, retaining 51 % of maximal activity at 30 ºC compared with only 29% activity for the *Piromyces* XI. This new enzyme represents a highly valuable addition to the *S. cerevisiae* molecular toolbox and shows promise for improved industrial conversion of carbohydrates.

This work was supported by the project FatVal PTDC/EAM-AMB/32506/2017 (POCI-01-0145-FEDER-032506), co-funded by the ERDF, through the COMPETE 2020, and by the FCT I.P. CBMA was supported by the "Contrato-Programa" UIDB/04050/2020 funded by the FCT I.P. PCS is recipient of a FCT PhD fellowship (SFRH/BD/140039/2018).