Bacterial Lytic Polysaccharide Monooxygenases (LPMOs) and their impact on deconstruction of lignocellulosic biomass for production of second generation ethanol

Thaís G. Silva*, Thamy L. R. Corrêa, Gonçalo A. G. Pereira.

Abstract
Lignocellulosic biomass is a renewable and low-cost source of energy, emerging as an alternative to the use of fossil fuels. The production of second generation ethanol (2G) is still considered onerous mainly due to the saccharification step. In this regard, Lytic Polysaccharide Monooxygenases (LPMOs) emerge as boosters of (hemi) cellulase activities helping to reduce the costs related to saccharification by decreasing the loading of canonical enzymes in reaction.

Key words:
LPMOs, Saccharification, Ethanol 2G.

Introduction

The second generation ethanol (2G) is an alternative to the use of fossil fuels as energy due to its renewable nature and the lower emissions of greenhouse gases in the atmosphere. However, its production is still considered onerous mainly due to the saccharification step that requires high dosage of enzymes to convert the polymers found in biomass into fermentable sugars. Enzymes currently classified as Auxiliary Activity 9 and 10 (AA9 and AA10) or Lytic Polysaccharide Monooxygenases (LPMOs) emerge as boosters of (hemi) cellulase activities reducing the costs of this step by decreasing the loading of canonical enzymes on reaction. AA10 are found in bacteria and presents a flat active site that helps the interaction with the crystalline portion of the polymer by a "histidine arm" that accommodates the copper ion, essential for catalysis [1].

Objective: Selection, cloning, purification, characterization and application of new sources of AA10 on lignocellulosic biomass deconstruction aiming the production of ethanol 2G.

Results and Discussion

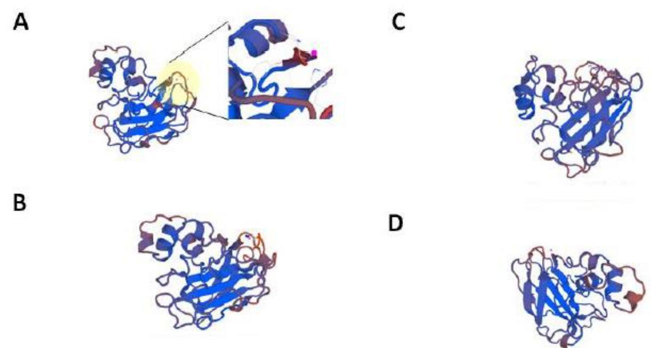
The search for new AA10 sequences was performed using the BLASTP tool (https://www.ncbi.nlm.nih.gov) employing four characterized (R1-R4) AA10 as references.

Chart 1. Identity of the proteins (%) using BLASTP tool

Microorganism	R1 (C1/C4)*	R2 (C1)*	R3 (C1/C4)*	R4 (C1)*
X	65	x	79	x
Y	58 (a)/ 32 (b)	x	x	x
Z	76	x	x	x

*(C1) and (C1/C4) refer to the oxidation sites.
The microorganism names were replaced by X, Y and Z given that they are target of patents.

Image 1. Structural identity (SWISS-MODEL) of putative AA10 from *X* (a), *Ya* (b), *Yb* (c) and *Z* (d) with AA10 from R1,R2,R3 and R4. The amino acids highlighted in red are regions with higher structural identity to the reference sequences.



In addition, the sequences *X, Y* and *Z* also present the Histidine (H) residue on the N-terminal portion involved with AA10 catalysis.

According to the results above, AA10 from *X, Y* and *Z* were considered the best candidates to be cloned.

Conclusions

Putative sequences of AA10 were prospected based on BLASTP and structural similarities with four references. These sequences were cloned and are currently being investigated regarding their boosting activities when added to commercial enzymatic cocktails.

Acknowledgement

[1].Vaaje-Kolstad, G.; Bjørge, W.; Horn, S.J.; Liu, Z.; Zhai, H.; Sørlie, M.; Eijsink, V.G.H. *Science* **2010**, *330*, 219–222