NEW THERMOPHILIC PROTEIN WITH XYLANASE ACTIVITY IN HOT SPRINGS

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1. INTRODUCTION

Cereal hemicellulose, called also arabinoxylan (AX) or pentosan, is a polysaccharide made up of a linear skeleton of xylose monomers linked by β-1,4 bonds with branches mainly due to L-arabinofuranose monomers that bind to oxygen of the C2 or C3, or even the oxygen of the C2 and C3 of the same xylose residue. Hemicellulose can be used to obtain different products depending on the degree of hydrolysis of the polysaccharide. The enzymes responsible for the hydrolysis of the xylan chain to xylose are generally called xylanases, the most important are the endo-1,4-β-xylanases, which with the β-xylosidase enzymes carry out the exhaustive hydrolysis of xylan to xylose, using the site of attack show in the Figure 1 (1).

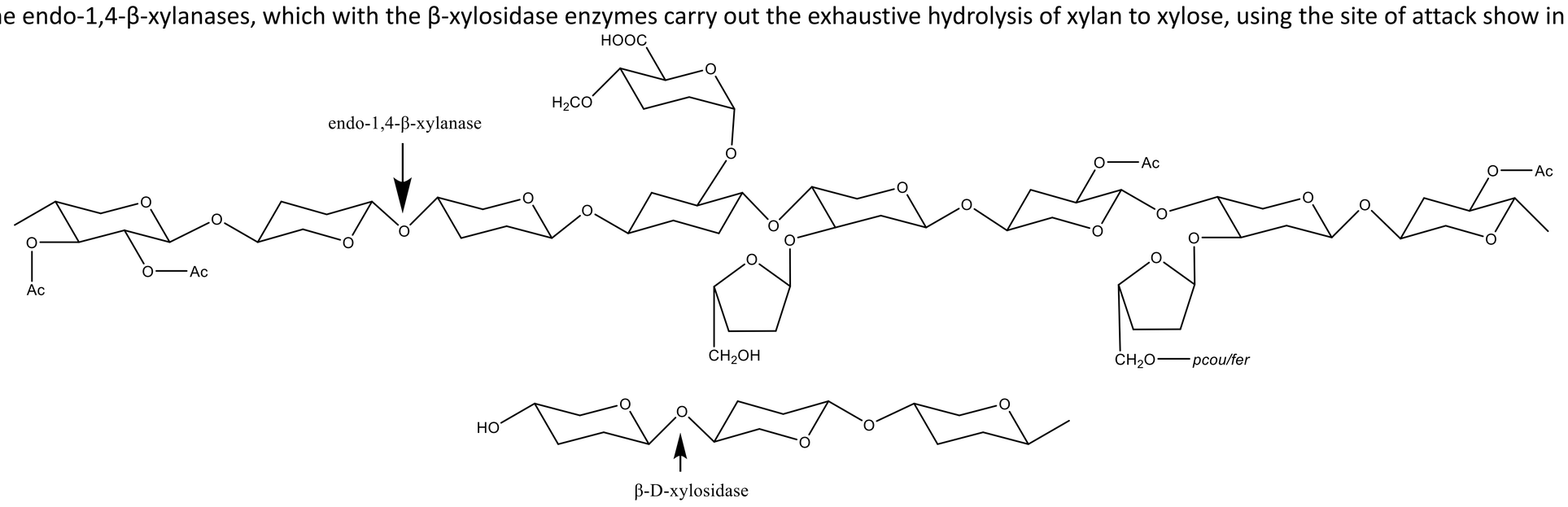


Figure 1. Structure of xylan and sites of attack by xylanolytic enzymes. The backbone of the substrate is composed of 1,4-β-linked xylose residues. Ac., Acetyl group; pcou., p-coumaric acid; fer., ferulic acid, and hydrolysis of xylo-oligosaccharide by β-xylosidase (2).

2. MAIN GOAL

The main objective of this work was to discover new thermoenzymes, mainly endo-xylanases and β-xylosidases.

3. RESULTS AND DISCUSION

The search for these new thermoenzymes was carried out by means of culture techniques and metagenomic libraries construction, from waters samples of two thermal springs, Burgas and Río Caldo (Figure 2 and 3) using as the single carbon source for enrichment cultures two varieties of wheat straw (Caaveiro and Castilla).

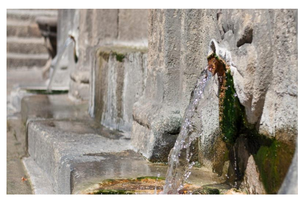
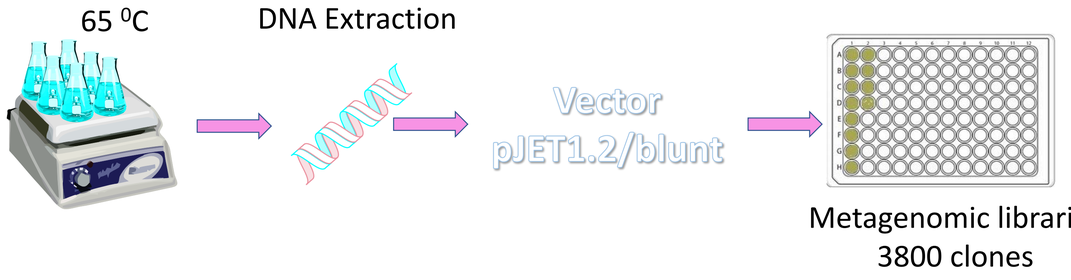


Figure 2. Burgas (Ourense) 67 ^{0}C

Figure 3. Río Caldo (Ourense) 77 ^{0}C

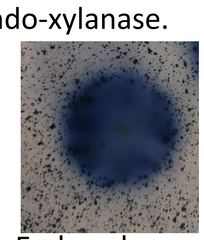
1) Isolation, DNA extraction and metagenomic libraries construction



Metagenomic libraries
3800 clones

2) Search for endo-xylanases and β-xylosidases in metagenomic libraries

The search for new thermoenzymes in metagenomic libraries was realized using different substrates. On the one hand, thermoenzymes endo-xylanases were detected used AZCL-Xylan substrate in LBA plates and on the other hand thermoenzymes β-xylosidase was detected using substrate fluorescence 4-methylumbelliferyl-β-D-xylopyranoside in LBA plates. Using this method was detected one thermophilic endo-xylanase.



Endo-xylanase

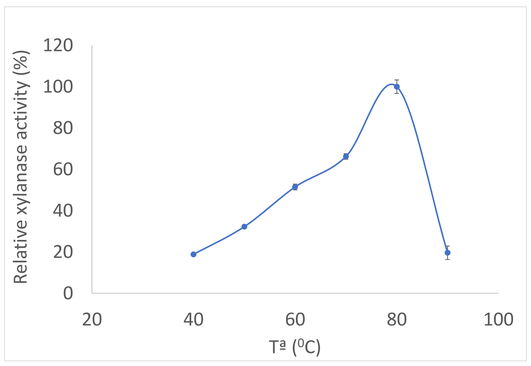
3) Expression of endo-xylanase

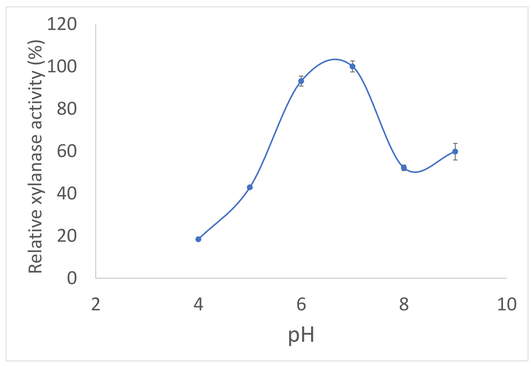
The xylanase was amplified directly from the metagenomic DNA with the specific primers and was cloned in the pDONR-221 vector using Gateway Technology (Invitrogen). From the gateway vector, the gene was shuttled into the his-tagged expression vector pDEST-527, using the Gateway LR recombination reaction (Invitrogen). The construction was transformed and expressed in One Shot BL21(DE3)(ThermoFisher) *E.coli*.

4) Activity assays

Endo-xylanase
Expressed in extracellular medium

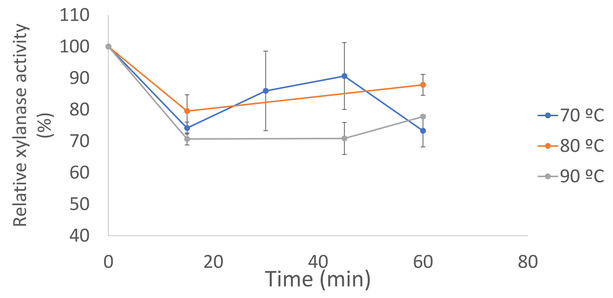
Using xylan as a substrate, the optimal temperature, pH and thermostability assays were determined for the enzyme expressed in the extracellular medium.





The optimum temperature of the enzyme at pH 6.5 using xylan as a substrate is 80 ^{0}C, while the optimum pH at 80 ^{0}C using the same substrate is 6.5.

The thermal stability of the enzyme was studied at 70 ^{0}C, 80 ^{0}C and 90 ^{0}C, during 1 h, using xylan as a substrate.



In relation to thermal stability, endo-xylanase was able to retain between 90%-70% of its maximum activity after 1 h incubation at 70, 80 and 90 °C.

4. CONCLUSIONS

One thermophilic endo-xylanase was found through functional metagenomics. Furthermore, this enzyme has ben expressed extracellularly and has a temperature optimum at 80 ^{0}C and a pH optimum of 6.5. Plus, this enzyme maintains a high relative activity at 70 ^{0}C, 80 ^{0}C and 90 ^{0}C, during 1 h.

5. FUTURE PERSPECTIVES

Once the pure endo-xylanase has been obtained, the kinetic study will be carried out, and finally, the enzymatic treatment conditions with xylanase and β-xylosidase obtained in this work will be designed to produce hydrolysates enriched in XOS and AXOS.

REFERENCES: (1) Izydorczyk, M. S.; Biliaderis, C. G. Studies on the Structure of Wheat-Endosperm Arabinoxylans. *Carbohydr. Polym.* **1994**, *24* (1), 61–71. https://doi.org/10.1016/0144-8617(94)90118-X. (2) Collins, T.; Gerday, C.; Feller, G. Xylanases, Xylanase Families and Extremophilic Xylanases. *FEMS Microbiology Reviews*. January 2005, pp 3–23. https://doi.org/10.1016/j.femsre.2004.06.005

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