



AMPLIFICATION, CLONING AND *IN SILICO* ANALYSIS OF A PUTATIVE XYLANASE FROM *ESCHERICHIA COLI*

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Introduction. In response to the increasing demand for biofuels, advanced biochemical processes using enzymes have been developed (e.g. the use of cellulases and xylanases to hydrolyze the cellulose and hemicellulose, respectively, to fermentable sugars for bioethanol production). However, the high cost of enzymes used as catalysts on biofuels production is the main drawback at industrial scale. The search of new enzymes with an increased catalytic activity is an alternative that could reduce costs. In attending to the search of solutions to this issue, the objective of this work was to amplify, clone and carry out the *in silico* analysis of a putative xylanase from *E. coli*.

Methods. Selection of the sequence and microorganism: Using the NCBI database a putative xylanase from *Escherichia coli* was selected. Primers design: The selected sequence was used to design the primers used for PCR amplification of the putative xylanase, the restriction site for *NotI* was introduced to the primers sequence (Forward Primer: Forward: 5'-ATG CCG CCG CAT GGA ACT TAT TGA A-3'; Reverse: 5'-ATG CCG CCG CTT ATT TAA ATA ATT T-3'). DNA extraction: The protocol proposed by Chen and Kuo (1) was employed to extract DNA from *E. coli*. Gene amplification: The sequence was amplified by PCR (MaxyGene, Gradient, Axygen, USA), using the following PCR program (1 cycle; 94°C, 5 min. 30 cycles; 94°C 1 min; 30°C, 1 min; 72°C, 2 min. 1 cycle; 72°C, 10 min). Cloning: pET-28a(+) vector was employed to clone the xylanase gene, and the resulting construction was introduced into the host microorganism, *E. coli* Rosetta (DE3). *In silico* analysis: For *in silico* analysis the following bioinformatic tools available online, were used; ProtParam, SOPMA, RSCB PDB, SwissModel, BLAST, and Clustal W2.

Results. The putative xylanase gene was amplified successfully (Figure 1), and PCR amplification showed the presence of a 1197 bp length fragment that agrees with the size expected (from the putative xylanase gene selected from NCBI). Once achieved the amplification of the putative gene, we proceeded to clone it on the vector pET-28a(+), and the resulting recombinant molecule, was employed to transform *E. coli* Rosetta (DE3). The transformation generates colonies of clones with the presence of the putative xylanase gene under control of the T7 promoter. *In silico* analysis of the xylanase sequence using the SwissModel application, generates a probable 3-D model of the putative enzyme. According to this 3-D structure (Figure 2), the template of a xylanase Z from *Clostridium thermocellum* was used to model the probable structure of

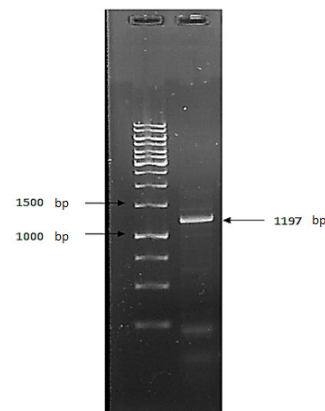


Figure 1. Amplification of the putative xylanase from *E. coli*

the putative enzyme. This probable protein has a typical β -glycanase fold which is characterized by an eight-stranded α/β -barrel, characteristic of the glycosyl hydrolase family 10. Using ClustalW2, we found that an Asp/His/Ser catalytic triad and a hydrophobic pocket present in the sequence of the xylanase Z from *C. thermocellum*, are conserved in the putative xylanase from *E. coli* (2).

Conclusions. Amplification of a DNA fragment associated to the synthesis of a putative xylanase from *E. coli*, was performed. The putative gene was cloned into the pET-28a(+) vector. *In silico* analysis of the putative enzyme showed similarities with the xylanase Z from *C. thermocellum*. Nevertheless, further studies are necessary.

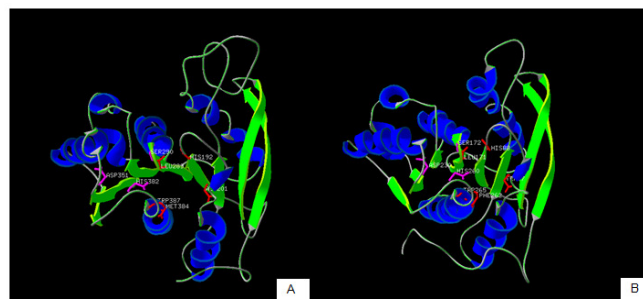


Figure 2. A) Modeled xylanase from *E. coli*. B) Xylanase Z from *Clostridium thermocellum*, used as template. In blue are highlighted the α -helices and in green the β -sheets. The magenta amino acids take part of the catalytic triad and the red ones correspond to the hydrophobic pocket.

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References.

1. Chen W, Kuo T, (1993) *Nucleic Acids Research* 21(9): 2260.
2. Schubot FD, Kataeva IA, Blum DL, Shah AK, Ljungdahl LG, Rose JP, Wang B-C, (2001) *Biochemistry* 40, 12524-12532.