FUNCTIONAL STUDY OF RECOMBINANT GH12 XILOGLUCANASES FOR XYLOGLUCAN HYDROLYSIS

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Introduction Biofuels, nowadays, is the focus of sustainable world. Therefore, studies propose the production of second generation ethanol, through enzymatic hydrolysis of sugar cane wastes. The cellulose hydrolysis is necessary in second generation ethanol output; these fibers are covered by other types of polysaccharides, then accessory enzymes become essential to cellulose hydrolytic attack. One of these polysaccharides is the xyloglucan, connected to cellulose fibers; xyloglucanases are needed to favor the access of cellulases.

Material and Methods Two xyloglucanases, assigned to family 12 of glycoside hydrolases (GH12), were cloned from Aspergillus clavatus (AclaXegA) and Aspergillus terreus (AtEglD). These genes were ligated to pEXPYRS vector and cloned in E. coli, followed by transformation in Aspergillus nidulans AT773 system aiming to reach high level of secretion. AclaXegA and AtEglD were secreted by A. nidulans, purified and sequenced by LC/MS-MS.

Results and Discussion Based on primary sequence, AclaXegA and AtEglD showed 46\% of identity and 65\% of similarity. β-sheets were the predominant secondary structure for both AclaXegA and AtEglD. The optimum pH and temperature for hydrolysis were 60°C/5.5 and 55°C/5.0 for AclaXegA and AtEglD, respectively. The AclaXegA was specific for xyloglucan hydrolysis, unlike, the activity of AtEglD was higher on linear substrate like beta-glucan than xyloglucan. Xyloglucan was hydrolysed to hepta- (XXXXG), octa- (XXXLG) and nonasaccharides (XLLG) by both AclaXegA and AtEglD. The AtEglD hydrolyzed beta-glucan releasing tri- (C3) and tetrasaccharides (C4) as major products.

Conclusion The creation of a library enzyme confers the opportunity to work in various sectors. The structural and functional understanding of new enzymes is essential for basic research and makes possible biotechnological manipulations to obtain high productivity of target proteins. These results for GH12 xyloglucanases highlight new insights on enzymes specificity. We are designing mutants of GH12 to try understand the structural features that define the high specificity of AclaXegA.

Keywords: GH12, Xyloglucanases, Xyloglucan, Enzimatic Hydrolysis, Aspergillus clavatus

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