Structure/function/properties relationships and application of a GH11 xylanase

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Background and aim: Xylanases are hemicellulolytic enzymes, which are responsible for the degradation of the heteroxylans constituting the lignocellulosic plant cell wall. Due to their variety, xylanases have been classified in glycoside hydrolase families GH5, GH8, GH10, GH11, GH30 and GH43 in the CAZy database. In this work, we focus on GH11 family, which is one of the best characterized GH families with bacterial and fungal members. GH11 xylanases have for a long time been used as biotechnological tools in various industrial applications and represent in addition promising candidates for future other uses.

Methods: Expression was done using *E. coli* and *Pichia pastoris*. The crystal structure of the XAn11 was solved at 1.14 A resolution.

Results: A new β-1,4-D endoxylanase (XAn11) belonging to the xylanase 11 family was purified to homogeneity from a newly soil-isolated *Aspergillus niger* US368 strain. The pure xylanase is a glycosylated monomer having a molecular mass of about 26 kDa. The gene encoding the XAn11 was cloned and sequenced. The XAn11 was found to be stable in a wide range of pH (3–9) and in presence of some detergents and organic solvents. A structural explanation of the difference between experimental and theoretical molecular mass as well as the stability of the enzyme against acidic pH was proposed by molecular modeling. The XAn11 cDNA was first cloned in pET-28a(+) and the recombinant plasmid was transformed in *Escherichia coli*. The His-tagged r-XAn11 was purified using Ni-NTA affinity and anion exchange chromatography. In the presence of 3 mM Cu^{2+}, the relative activity of the His-tagged r-XAn11 was enhanced by 54%. This is the first work reporting that copper is a strong activator for xylanase activity making this enzyme very attractive for future industrial applications. The cDNA encoding the β-1,4-endoxylanase of *Aspergillus niger* US368 was also cloned and expressed in *Pichia pastoris* under the constitutive GAP promoter. The maximum activity obtained was 41 U mL^{-1}, which was about 3-fold higher than that obtained with the native species. r-XAn11-His6 (recombinant xylanase) was used *in vitro* digestion of barley and wheat bran leading to a decrease of the viscosities and an increase of the reducing sugars and total sugars contents. In this study, the crystal structure of the XAn11 was solved at 1.14 A resolution.

Conclusion: In this study, a decrease in water absorption, an increase in dough rising and improvements in volume and specific volume of the bread were recorded. The r-XAn11-His6was also used in in vitro digestion of barley and wheat bran leading to a decrease of the viscosities and an increase of the reducing sugars and total sugars contents.

Keywords: GH11 Xylanase, *Aspergillus niger*, heterologous expression, crystal structural industrial application.