

Characterization of new bacterial glycoside hydrolases isolated from agricultural soils using a functional metagenomic approach

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Microorganisms play key roles in soil ecosystem functioning, notably through their ability to degrade plant cell wall polymers. For this, bacteria and fungi produce various enzymes such as cellulases, xylanases, glucosidases, esterases or laccases. Finding new enzymes hydrolyzing cellulose, hemicellulose or lignin is not only interesting for a better understanding of the roles of the soil microflora still largely unknown but these enzymes are also useful for various biotechnological applications such as the production of renewable energy from lignocellulosic material. So here, we used a functional metagenomic approach to isolate new bacterial β -glucosidases, which were then biochemically characterized. The new enzymes were identified by functional analysis of agricultural-soil metagenomic libraries hosted in *Escherichia coli* and screened on medium containing esculin. After sequence analysis and preliminary estimation of the activity of the new β -glucosidases using p-nitrophenol derivatives on intact bacterial cells, the coding sequences of three of them were cloned into a bacterial expression vector so as to overproduce and purify them by affinity chromatography. The chosen enzymes show only 52-64% sequence identity to known family 3 (GH3) or 1 (GH1) glycoside hydrolases of different phyla (Actinobacteria, Acidobacteria and Proteobacteria). Analysis of the *E. coli* cells expressing each of them revealed that both GH1 proteins (ASEsc9 and ASEsc10) are thermophilic enzymes more active at mildly acidic to neutral pH while the GH3 enzyme (ASEsc6) is an alkaline, mesophilic, β -glucosidase also displaying xylosidase activity. Their coding sequences have been cloned in fusion with a carboxy-terminal His-tag and placed under the control of the IPTG-inducible promoter of the pET-30b vector. The proteins will be overproduced and purified for further characterization.