Glycosylation Influences the Enzymatic Activity of the Recombinant Xyn3 from *Trichoderma harzianum* expressed in *Pichia pastoris*

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Xylanases are used in several biotechnological processes, primordially for biopulping and biobleaching in paper industries. However, these enzymes are showing increasing importance as accessory enzymes for bioethanol production. In this work, a cDNA encoding an endoxylanase was cloned from an induced cDNA library of *Trichoderma harzianum*. This xylanase showed similarity with the xyn3 from *T. reesei*, also presenting a N-glycosylation site at N-terminus of the mature enzyme. The xyn3 ORF from *T. harzianum* was cloned in the expression vector pGAPZαA, substituting the vector signal peptide by the native signal peptide. The recombinant plasmid was integrated into *Pichia pastoris* KM71H genome. The recombinant protein was expressed in two majority forms, one with a molecular mass of 35 kDa (non-glycosylated) and the other with about 60 kDa (glycosylated). Both forms showed xylanolytic activity in a zymogram test. Biochemical assays using dinitrosoalicylic acid (DNS) exhibited the highest activity around 40°C and pH 6.5. The glycosylation was not important in the thermal stability and both forms of the recombinant enzyme presented 50% of residual activity after 35 hours of incubation at 35°C, in absence of substrate. However, the glycosylation played important role in the enzymatic kinetics, showing a catalytic efficiency twice higher of glycosylated form, in comparison with the non-glycosylated one. This result illustrates the importance in using eukaryotic expression system to study enzymes from filamentous fungi, and also presents an attractive characteristic for the N-glycosylated xyn3 from *T. harzianum*.

Keywords: Xylanase, *Trichoderma harzianum*, N-glycosylation.
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