PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF A XYLANASE FROM *Cellulomonas uda* HETEROLOGOUS EXPRESSED IN *Pichia pastoris* BY INDUCTION WITH METHANOL.

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Introduction. Xylanases are enzymes capable of degrading xylan. They have important applications in industry due to its enormous potential to transform lignocellulosic materials, specifically hemicellulose[1]. *Pichia pastoris* is a methylotrophic yeast capable of producing significant quantities of recombinant protein[2]. In the absence of glucose, this yeast is able to use methanol as sole carbon source[3]. We have previously reported the cloning and expression of the *xyn11A* xylanase gene in *E. coli*[4]; however, most of the protein (~ 60%) was found in the insoluble inclusion body fraction of the bacterial cell lysate. Likewise we have reported the expression of the Xyn11A xylanase from *Cellulomonas uda* in *P. pastoris* by induction with methanol. The aim of this work was to purify and biochemically characterize the recombinant xylanase expressed in *Pichia pastoris*.

Methods

Production of extracellular recombinant xylanase in shake flask. Cells of *P. pastoris* harboring the plasmid pPICZαB–*xyn11A* were cultivated in two stages. First, cells were growth in YPD and then in BMMY medium, containing 0.5% methanol as carbon source, to induce the expression of the recombinant enzyme.

Xylanase purification. Xyn11A was purified from the culture supernatant of *P. pastoris*/pPICZαB–*xyn11A* cells by anion exchange chromatography, using a Unosphere Q (Bio-Rad) column. Absorbed proteins were eluted from the column with a linear gradient of KCl (25 mM-1 M). Fractions with xylanase activity were pooled and analyzed by 10% SDS-PAGE.

Biochemical characterization of recombinant xylanase Xyn11A. The effect of pH (4 to 8), temperature (30 to 75°C), metal ions (1,3 and 5 mM) and substrate concentration (birchwood xylan, from 1 to 10 mg/ml) on the enzymatic activity of Xyn11A was undertaken by individually changing conditions of the xylanase activity assay.

Results

The recombinant xylanase Xyn11A was extracellularly expressed in P. pastoris and purified from the culture supernatant by one step of anion exchange chromatography. 10% SDS-PAGE analysis of purified enzyme showed a single band of 45 kDa with a yield of 80.38% and specific activity of 874.73 U/mg.

Xyn11A showed optimal activity at pH 6.5 and 50°C but was active in a broad pH interval (5-7.5), retaining above 50% of the maximum activity. Drastic reduction in enzyme activity (to 40% of the maximum activity) occurred at 65-75°C.

Thermal stability studies were carried out by pre-incubating the enzyme at 40, 50 and 60°C. Xyn11A was sensitive to 60°C, since the half life was reached after 1.5 min of incubation, but at 40°C the enzyme retained 65% of its original activity after 77 h at 40°C.

The effect of some metal ions and the chelating agent EDTA on the enzymatic activity of Xyn11A was determined. Metal ions (Hg^{++}, Cu^{++}, Li^{++}, Zn^{++}, Ca^{++}, Mg^{++}, Fe^{++}, Ni^{++}, Na^{+}, Mn^{++}) at final concentrations of 1, 3 or 5 mM were individually tested; however no difference was found between the results obtained at the different concentrations assayed. Hg^{++} drastically inhibited xylanase activity, suggesting the existence of thiol groups at the active site of the enzyme. Mn^{++} decreased in 34.45% the activity of Xyn11A. None of the metal ions tested increased the activity of Xyn11A. EDTA decreased the xylanolytic activity in 18.62% suggesting that a few metals are required for the enzymatic activity of Xyn11A.

The concentration of birchwood xylan as the substrate was varied from 1 to 10 mg/ml in the reaction mixture. Inicial reaction rates versus substrate concentration shows that the enzyme obeyed Michealis-Menten Kinetics and had K_m of 2.3 mg/ml and V_{max} of 3500 U/mg.

Conclusions

Xyn11A was extracellular expressed in *P. pastoris* and the enzyme was purified by a single step of anion exchange chromatography with good purification yield (80%). The enzyme showed a molecular weight of 45 kDa. Xyn11A may be appropriate for applications in the food industry for animals and for the pretreatment of lignocellulosic wastes in the production of bioethanol because it is active in a wide range of pH 5 to 7.5 and is stable below 50 °C.

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References.

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