Title: Expression of a xylanase in *Komagataella phaffii* for biochemical characterization and assessment of industrial applications

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Abstract

Xylan, a component of plant cell walls, is composed of β-1, 4-linked xylose residues with side branches containing other monosaccharides. The main chain of xylan can be hydrolyzed by xylanases; which have applications in industry, such as extraction and clarification of fruit juices, processing of vegetable oil, winemaking, brewing, and baking. Enzyme production for biotechnological applications is a market expected to exceed US$6.2 billion worldwide by 2020. Xylanases can be produced by different microorganisms, and each enzyme has different biochemical characteristics. The choice of host for enzyme production is important because it can affect protein activity and yield. *Komagataella phaffi* is an efficient expression system. It can produce post-translationally-modified proteins, and also secrete them into the extracellular medium, facilitating protein recovery and purification. In this work, the transcriptome of a fungus from the Brazilian biodiversity was mined for sequences encoding putative xylanases; and four of these sequences were selected for expression in *K. phaffi*. Here we describe the expression of a xylanase named Xnd01. The strategy used for enzyme production was gene synthesis. Once the construct was obtained, it was propagated in *E.coli* so that the correctness of the construct was verified by restriction enzyme digests. After this, the plasmid was linearized with restriction enzymes, and the DNA was transformed into *K. phaffi* by electroporation. Putative transformed colonies were selected on YPDS plates supplemented with the antibiotic zeocin. A total of 21 putative transformants were obtained. These were then inoculated in 7 mL of YPD with zeocin, and cultures were tested for xylan activity after 72 h. Larger-volume cultures (250 mL) of one clone were grown and the supernatant containing the secreted xylanase was subjected to purification in an Akta Pure chromatography system. Enzymatic activity was determined using 7% xylan as substrate. Xylanase activity of 13.7 IU/mL was detected by the DNS method (5-dinitrosalicylic acid). SDS-PAGE and Western blotting were used to determine the purity of the xylanase. The optimum pH of Xnd01 was 5,6 and the optimum temperature was 55 °C. We will present the biochemical characterization of this xylanase for thermostability and enzyme kinetics. Possible industrial applications for this enzyme will be discussed.