HETEROLOGOUS EXPRESSION OF A LIPASE GENE FROM THE BASIDIOMICETE FUNGUS *Bjerkandera adusta* UAMH 8525 IN *Pichia pastoris*

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Introduction. The interest in lipases arises due to their ability to catalyze the hydrolysis of triacylglycerols and the synthesis of esters from glycerol and long-chain fatty acids under certain conditions (1). They are widely used in the dairy and oil chemical industries, in the synthesis of structured triglycerides, surfactants, pharmaceuticals and agrochemicals, and in polymers and household detergents (2). We report the cloning and sequencing of the genomic DNA and cDNA of a lipase gene from *Bjerkandera adusta* UAMH 8258 and its expression in *P. pastoris* X-33.

Methods. A cDNA library of *B. adusta* was constructed and more than 300 clones were sequenced. One of these showed significant homology with lipase/esterase genes from fungi. This sequence was then amplified through PCR from a sample of cDNA and genomic DNA of *B. adusta* grown in minimum medium with wheat straw as a carbon source. The fragment was then subcloned in vector pPICZαA, and was transformed *Pichia pastoris* strain X-33. The recombinant strain was grown in 50 ml of BMMY medium (YNB1.34%, yeast extract 1%, peptone 2%, biotin $4x10^{-5}$, methanol 0.5% and 100 mM potassium phosphate, pH 6.0) for induction. Cells were grown for 72 hours to recover supernatant by centrifugation (2000 × g for 10 min at 4°C), that was kept at 4°C until further use. Molecular weight estimation of recombinant lipase was performed resolving 20 μg of protein in 10% SDS-polyacrylamide gel.

Results. The lipase cDNA product was cloned and its sequence confirmed. The analysis of the 967 bp clone suggested that it encodes lipase with homology to the family of esterase/lipase. Lipase genomic DNA sequence of 1234 pb includes three introns (Fig 1). Expression in *Pichia pastoris* X-33 secreted the lipase into the culture supernatants; protein gels showed that the lipase molecular weight is approximately 37 KDa (34.4 KDa expected from the lipase plus 2.5 KDa corresponding to polyhistidine tag and the *myc* epitope) (Fig 2).

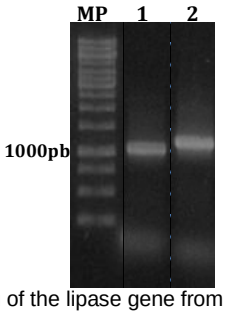

Fig.1 Cloning of the lipase gene from cDNA and genomic DNA. Lane MP: Marker. Lane 1: Amplification from cDNA. Lane 2: Amplification from DNA genomic.

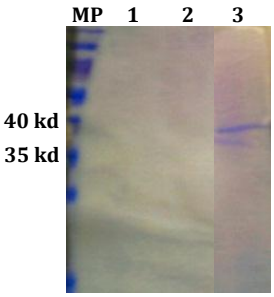

Fig 2 SDS-PAGE MP: Marker. Lane 1: *Pichia pastoris* wild-type. Lane 2: *Pichia pastoris*/pPICZαA. Lane 3: Recombinant *Pichia pastoris*/pPICZαA-Lipase.

Currently substrate specificity and other biochemical parameters are being explored.

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