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Expression of Pullulanase from Thermus Thermophilus HB8 in Pichia Pastoris and its Characterization

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Extended Abstract

Pullulanase is one of the important debranching enzymes. Its type I is used usually in the saccharification step of starch to catalyse the hydrolysis of the α-D-1,6 glucosidic linkages in amylopectin.

The objective of this study was to investigate the feasibility of extracellular production of a thermoduric pullulanase from *Thermus thermophilus* HB8 by *Pichia pastoris* MutS strains. Firstly, six secretion signal sequences were tested for extracellular pullulanase accumulation. The expression vector containing the alpha-factor –Kex-Ste of *Saccharomyces cerevisiae* (pD912-AKS-19) was able to lead to some extracellular pullulanase accumulation clearly detected at the 5th day of methanol-driven induction (0.14 U/ml). However, more than 98% of the pullulanase was accumulated intracellularly, contrary to expectations. To enhance the excretion of the recombinant pullulanase, natural osmolytes (proline, guanidine, betaine and K-glutamic acid), and Triton X-100, were tested. The production of extracellular pullulanase was increased more than 50-fold by adding K-glutamic acid at the final concentration of 0.4% (w/v) five hours before induction, and by adding Triton X-100 48h after induction. Afterwards, the effect of temperature and pH during induction on enzyme levels was evaluated. To characterize the recombinant pullulanase, a semi-purification protocol was firstly applied, made up of two steps: thermal treatment and centrifugation. The optimal pH and temperature of the recombinant pullulanase were, respectively, 6.0 and 70°C. 50% of the pullulanase activity was recovered after incubation at 70°C for 60 min.