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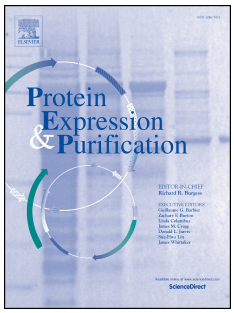

Biochemical characterization of chitinase A from *Bacillus licheniformis* DSM8785 expressed in *Pichia pastoris* KM71H

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Protein expression and purification

Biochemical characterization of chitinase A from *Bacillus licheniformis* DSM8785 expressed in *Pichia pastoris* KM71H

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ABSTRACT

Chitin is an abundant biopolymer found mainly in the exoskeleton of crustaceans and insects. The degradation of chitin using chitinases is one way to address the accumulation of chitin waste streams in the environment, and research has therefore focused on the identification, improvement and expression of suitable enzymes. Here we describe the production, purification and characterization of *Bacillus licheniformis* chitinase A in the *Pichia pastoris* expression system. Optimal enzyme activity occurred at pH 4.0–5.0 and within the temperature range 50–60°C. With colloidal chitin as the substrate, the K_m (2.307 mM) and V_{max} (0.024 mM·min^{-1}) of the enzyme were determined using a 3,5-dinitrosalicylic acid assay. The degradation products of colloidal chitin and hexa-*N*-acetylchitohexaose were compared by thin-layer chromatography. The activity of the glycosylated enzyme produced in *P. pastoris* was compared with the *in vitro* deglycosylated and aglycosylated version produced in