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# Purification and Characterization of a Novel Extracellular Chitinase from Bacillus licheniformis LHH100

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**Abstract :** Chitin, a linear 1, 4-linked N-acetyl-d-glucosamine (GlcNAc) polysaccharide is the major structural component of fungal cell walls, insect exoskeletons and shells of crustaceans. It is one of the most abundant naturally occurring polysaccharides and has attracted tremendous attention in the fields of agriculture, pharmacology and biotechnology. Each year, a vast amount of chitin waste is released from the aquatic food industry, where crustaceans (prawn, crab, Shrimp and lobster) constitute one of the main agricultural products. This creates a serious environmental problem. This linear polymer can be hydrolyzed by bases, acids or enzymes such as chitinase. In this context an extracellular chitinase (ChiA-65) was produced and purified from a newly isolated LHH100. Pure protein was obtained after heat treatment and ammonium sulphate precipitation followed by Sephacryl S-200 chromatography. Based on matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/MS) analysis, the purified enzyme is a monomer with a molecular mass of 65,195.13 Da. The sequence of the 27 N-terminal residues of the mature ChiA-65 showed high homology with family-18 chitinases. Optimal activity was achieved at pH 4 and 75◦C. Among the inhibitors and metals tested p-chloromercuribenzoic acid, N-ethylmaleimide, Hg2+ and Hg + completelyinhibited enzyme activity. Chitinase activity was high on colloidal chitin, glycol chitin, glycol chitosane, chitotriose and chitooligosaccharide. Chitinase activity towards synthetic substrates in the order of p-NP-(GlcNAc) n (n = 2–4) was p-NP-(GlcNAc)2> p-NP-(GlcNAc)4> p-NP-(GlcNAc)3. Our results suggest that ChiA-65 preferentially hydrolyzed the second glycosidic link from the non-reducing end of (GlcNAc) n. ChiA-65 obeyed Michaelis Menten kinetics the Km and kcat values being 0.385 mg, colloidal chitin/ml and5000 s−1, respectively. ChiA-65 exhibited remarkable biochemical properties suggesting that this enzyme is suitable for bioconversion of chitin waste.

**Keywords :** Bacillus licheniformis LHH100, characterization, extracellular chitinase, purification
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