PRODUCTION OF BIOPOLYMER, CHITOSAN, BY ENZYMATIC REACTION USING RECOMBINANT CHITIN DEACETYLASE EXPRESSED IN *PICHIA PASTORIS*

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Chitin deacetylase catalyzes the hydrolysis of N-acetoamido bonds of chitin by converting it to chitosan. Chitin deacetylase gene, *CDA2*, from *Saccharomyces cerevisiae* has been cloned and expressed in *Pichia pastoris* using the pICZ A vector™. The recombinant protein, Cda2p, containing a C-terminal His6 tag was expressed in high level and was purified to homogeneity from the soluble fraction by single step purification in a Ni^{+} chromatographic column. The purified protein exhibited an apparent molecular mass of approximately 37kDa as judged by SDS-PAGE and its identity was confirmed by MALDI/TOF analysis. The degree of deacetylation of natural chitin or N-acetylchitooligosaccharides was evaluated by determination of the amine released using o-phtaldehyde reagent (OPA). Recombinant Cda2p was able to deacetylated chitooligosaccharides containing two, three or four N-acetyl D-glicosamine residues. When tetraose chitoologossacharide was used as substrate the optimum temperature for enzyme activity was 50°C, the pH optimum, 8.0, Km of 100mM and Vmax of 6.6 nmoles/min. The recombinant protein was also able to deacetylate shrimp crystalline chitin with a specific activity of 134,3U/min x mg protein. The physical, chemical, electronic characteristics and structural information of the new polymers will be achieved by NMR-H^{+}, providing detailed information on the three-dimensional structure of chitosan to be used in different biomedical applications.
Financial support: Petrobrás, FINEP, FAPERJ, FUJB and CAPES.