

# Biochemical characteristics of chitosanase from the Indonesian *Bacillus licheniformis* MB-2

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## Abstract

*Bacillus licheniformis* MB-2, isolated from a hot spring water in Manado, Indonesia, secreted a unique chitosanase. Media consisted of 0.24% chitosan, 0.25% casiton, 1% MgSO<sub>4</sub>, 1.4% K<sub>2</sub>HPO<sub>4</sub>, 0.02% CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.002% FeSO<sub>4</sub>·7H<sub>2</sub>O (w/v) was used for enzyme production. Purification of the enzyme through the hydrophobic interaction chromatography system (butyl Sepharose 4 FF) resulted in two major active fractions; the F2 fraction was shown as a single band at both sodium dodecyl sulfate-polyacrylamide gel electrophoresis and zymogram analysis with apparent molecular mass of 75 kDa. The enzyme worked best at 70°C and pH between 6.0 and 7.0. When incubated at 70, 80, and 90°C, the t<sub>1/2</sub> values were 26.56, 18.44, and 16.74 min, respectively with the k constant being at 0.026, 0.037, and 0.04/min. When heated at 90°C, the enzyme retained its activity up to 8 h in the presence of 1mM MnCl<sub>2</sub>. The enzyme's activity was unaffected by the presence of 1 M NaCl and 6 M urea but was decreased by 2 M of guanidine hydrochloride. Albeit the enzyme did not degrade colloidal and glycol chitin, it hydrolyzed glycol chitosan up to 0.8% and colloidal chitosan up to 11%. The

85% deacetylated (DDA) soluble chitosan was the most susceptible to this enzyme, followed by 90% and 100% DDA chitosan. The  $K_m$  values of the 85, 90, and 100% DDA soluble chitosans were found as 0.23, 0.24, and 0.58 mg/mL, whereas the  $V_{max}$  values were 843, 668, and 261 U/mg, respectively. The hydrolysis products of F2 chitosanase at 24 h incubation (70°C) were pentasaccharide (GlcN)<sub>5</sub> and hexasaccharide (GlcN)<sub>6</sub>. The preliminary test showed inhibitory effect of chitooligosaccharides resulted from enzymatic degradation toward *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus*.