A β-Mannanase from *Bacillus subtilis* B36: Purification, Properties, Sequencing, Gene Cloning and Expression in *Escherichia coli*

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MANB36, a secrete endo-β-1,4-D-mannanase produced by *Bacillus subtilis* B36, was purified to homogeneity from a culture supernatant and characterized. The optimum pH value for the mannanase activity of MANB36 is 6.4 and the optimum temperature is 50 °C. The enzyme activity of MANB36 is remarkably thermostable at 60 °C and the specific activity of MANB36 is 927.84 U/mg. Metal cations (except Hg^{2+} and Ag^{+}), EDTA and 2-mercaptoethanol (2-ME) have no effects on enzyme activity. This enzyme exhibits high specificity with the substituted galactomannan locust bean gum (LBG). The gene encoding for MANB36, *manB36*, was cloned by PCR and sequenced. *manB36* contains a single open reading frame (ORF) consisting of 1104 bp that encodes a protein of 367 amino acids. The predicted molecular weight of 38.13 kDa, calculated by the deduced protein of the gene *manB36* without signal peptide, coincides with the apparent molecular weight of 38.0 kDa of the purified MANB36 estimated by SDS-PAGE. The mature protein of MANB36 has been expressed in *Escherichia coli* BL21 and the expressed mannanase has normal bioactivity.

Key words: β-Mannanase, *Bacillus subtilis*