Exhaustive Study of the Novel Hyper Alkalophil, Thermostable, and Chelator Resistant Metalloprotease

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Abstract

Our newly discovered metalloprotease, designated as ALP NS12 was selected using gelatin agar plates with incubation at 100 °C. Subcloning of the fragments in to pUC118 to make E. coli HB101 (pPEMP41NS) with following two steps chromotography using diethylaminoethyl sepharose (DEAE-sepharose) and Sephadex G-100 columns to purify 97% of the expressed enzyme was performed. Although activity of immobilized ALP NS12 on glass surface was established at temperatures between 70 and 120 °C and pH ranges 4.0-13.0, the optimum temperature and pH were achieved at 100 °C and 11.0, respectively. Enhancement of enzyme activity was obtained in the presence of 5 mM MnCl₂ (81 %), CaCl₂ (58 %), FeCl₃ (175 %), MgCl₂ (94 %), ZnCl₂ (412 %), NiCl₂ (88 %), NaCl (239 %), and Na lactate (81 %) while inhibition was observed with EDTA (5 mM), PMSF (3 mM), urea (8 M), and SDS (1 %) at 95, 37, 33, and 42 °C, respectively. Consequently, the enzyme was well analyzed using x-ray crystallography and protein modeling. ALP NS12 can be applied in industrial processes at extreme temperatures and under highly basic conditions, chelators, and detergents.