

A thermoalkaliphilic lipase of *Geobacillus* sp. T1

Abstract

A thermoalkaliphilic T1 lipase gene of *Geobacillus* sp. strain T1 was overexpressed in pGEX vector in the prokaryotic system. Removal of the signal peptide improved protein solubility and promoted the binding of GST moiety to the glutathione-Sepharose column. High-yield purification of T1 lipase was achieved through two-step affinity chromatography with a final specific activity and yield of 958.2 U/mg and 51.5%, respectively. The molecular mass of T1 lipase was determined to be approximately 43 kDa by gel filtration chromatography. T1 lipase had an optimum temperature and pH of 70°C and pH 9, respectively. It was stable up to 65°C with a half-life of 5 h 15 min at pH 9. It was stable in the presence of 1 mM metal ions Na⁺, Ca²⁺, Mn²⁺, K⁺ and Mg²⁺, but inhibited by Cu²⁺, Fe³⁺ and Zn²⁺. Tween 80 significantly enhanced T1 lipase activity. T1 lipase was active towards medium to long chain triacylglycerols (C10–C14) and various natural oils with a marked preference for trilaurin (C12) (triacylglycerol) and sunflower oil (natural oil). Serine and aspartate residues were involved in catalysis, as its activity was strongly inhibited by 5 mM PMSF and 1 mM Pepstatin. The T_m for T1 lipase was around 72.2°C, as revealed by denatured protein analysis of CD spectra.

Keyword: *Geobacillus* sp., Thermoalkaliphilic, Overexpression, Purification, Thermostable lipase