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