High Level Expression of Thermostable Lipase from Geobacillus sp. Strain T1

ABSTRACT

A thermostable extracellular lipase of Geobacillus sp. strain T1 was cloned in a prokaryotic system. Sequence analysis revealed an open reading frame of 1,251 bp in length which codes for a polypeptide of 416 amino acid residues. The polypeptide was composed of a signal peptide (28 amino acids) and a mature protein of 388 amino acids. Instead of Gly, Ala was substituted as the first residue of the conserved pentapeptide Gly-X-Ser-X-Gly. Successful gene expression was obtained with pBAD, pRSET, pET, and pGEX as under the control of araBAD, T7, T7 lac, and tac promoters, respectively. Among them, pGEX had a specific activity of 30.19 Umg−1 which corresponds to 2927.15 Ug−1 of wet cells after optimization. The recombinant lipase had an optimum temperature and pH of 65°C and pH 9, respectively. It was stable up to 65°C at pH 7 and active over a wide pH range (pH 6–11). This study presents a rapid cloning and overexpression, aimed at improving the enzyme yield for successful industrial application.

Keyword: Geobacillus sp., thermostable lipase, Glutathione S-transferase (GST) fusion protein, cloning; overexpression