A newly isolated thermostable lipase from Bacillus sp.

ABSTRACT

A thermophilic lipolytic bacterium identified as Bacillus sp. L2 via 16S rDNA was previously isolated from a hot spring in Perak, Malaysia. Bacillus sp. L2 was confirmed to be in Group 5 of bacterial classification, a phylogenically and phenotypically coherent group of thermophilic bacilli displaying very high similarity among their 16S rRNA sequences (98.5–99.2%). Polymerase chain reaction (PCR) cloning of L2 lipase gene was conducted by using five different primers. Sequence analysis of the L2 lipase gene revealed an open reading frame (ORF) of 1251 bp that codes for 417 amino acids. The signal peptides consist of 28 amino acids. The mature protein is made of 388 amino acid residues. Recombinant lipase was successfully overexpressed with a 178-fold increase in activity compared to crude native L2 lipase. The recombinant L2 lipase (43.2 kDa) was purified to homogeneity in a single chromatography step. The purified lipase was found to be reactive at a temperature range of 55–80 °C and at a pH of 6–10. The L2 lipase had a melting temperature (Tm) of 59.04 °C when analyzed by circular dichroism (CD) spectroscopy studies. The optimum activity was found to be at 70 °C and pH 9. Lipase L2 was strongly inhibited by ethylenediaminetetraacetic acid (EDTA) (100%), whereas phenylmethylsulfonyl fluoride (PMSF), pepstatin-A, 2-mercaptoethanol and dithiothreitol (DTT) inhibited the enzyme by over 40%. The CD spectra of secondary structure analysis showed that the L2 lipase structure contained 38.6% α-helices, 2.2% ß-strands, 23.6% turns and 35.6% random conformations.

Keyword: Bacillus sp. strain L2; Thermostable lipase; Cloning; Sequencing; Molecular expression; Characterization.