RECOMBINANT Anoxybacillus flavithermus T1 ESTERASE/LIPASE: OPTIMIZATION OF EXPRESSION AND RECOVERY

Laura-Mihaela Chiş¹, Monica Hriscu¹, Flore Chirilă², Iulia Lupan³, Monica Toşa¹, Florin Dan Irimie¹*

¹Babeș-Bolyai University, Faculty of Chemistry and Chemical Engineering, Dept. of Biochemistry and Biochemical Engineering, 11 Arany Janos St., RO-400028 Cluj-Napoca, Romania
²Dept. of Paraclinical Education, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, 3-5 Calea Mănăștur, RO-400372 Cluj-Napoca, Romania
³Babeș-Bolyai University, Institute for Interdisciplinary Experimental Research, Molecular Biology Center, 42 A.T. Laurian St., RO-400271 Cluj-Napoca, Romania

Abstract

We have previously described the molecular cloning and expression in an Escherichia coli system and the characterization of a thermostable esterase/lipase from Anoxybacillus flavithermus T1. We herein report on the optimization of the expression process. When using isopropyl-β-D-thiogalactopyranoside (IPTG) for induction, the highest protein yield was obtained at 30°C, with 0.4 mM IPTG and 1 h induction time. Similar results were obtained at 37°C, but with a higher IPTG concentration (5 mM) and after 8h of induction, which makes the former a better option in terms of cost and time-effectiveness. Better results yet were attained with lactose, a very attractive option, given its high availability, low cost and low toxicity to the host cells. Recovery of the active enzyme from the periplasmic space was highest with a lysis buffer which combines osmotic shock with a membrane destabilization effect (Tris-sucrose/EDTA 1 mM/MgSO₄ 5 mM). The freeze-thaw treatment yielded similar results, while treatment with 1% organic solvent (chloroform or DMSO), while effective in permeabilization of the cell membrane, exerted a certain inhibitory effect upon the enzyme.

Key words: Anoxybacillus flavithermus T1 esterase/lipase, IPTG, lactose, membrane destabilization, periplasmic fraction

Received: July 2012, Revised final: October 2012, Accepted: November 2012

* Author to whom all correspondence should be addressed: e-mail: irimie@chem.ubbcluj.ro; Phone: +40(0)264 591-897; Fax: +40(0)264 190-818