Chiş, L.M., Hriscu, M., Chirilă, F., Lupan, I., Toşa, M., Irimie, F.D. (2012):
Recombinant *Anoxybacillus flavithermus* T1 esterase/lipase: optimization of expression and recovery,
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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-efectiveness. 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/$MgSO_4$ 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

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