Laura Chiș, Monica Hriscu, Adriana Bica, Monica Toșa, Gergely Nagy, Gergely Rona, Beata Vertessy, Florin Dan Irimie (2013): Molecular cloning and characterization of a thermostable esterase/lipase produced by a novel *Anoxybacillus flavithermus* strain
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Abstract
A thermophilic strain producing an extracellular esterase/lipase was isolated from a hot spring in Tășnad, Romania, and was identified phenotypically and by 16S rDNA sequencing as *Anoxybacillus flavithermus* (GenBank ID: JQ267733). The gene encoding the putative carboxyl esterase (GenBank ID: JX494348) was cloned by direct PCR amplification from genomic DNA. The protein, consisting of 246 amino acids and having a predicted molecular weight of 28.03 kDa, is encoded by an ORF of 741 bps. Expression was achieved in *Escherichia coli* and a recombinant protein with esterolytic activity and estimated molecular weight of 25 kDa was recovered and purified from the periplasmic fraction by IMAC. The purified enzyme, most active at 60–65°C and in the near-neutral range (pH 6.5–8), displayed a half-life at 60°C of about 5 h. Est/Lip displayed a relative tolerance to methanol, DMSO, acetonitrile, and low detergent concentrations (SDS, Triton) increased its thermostability. Highest activity was attained with *p*-nitrophenyl butyrate, but the enzyme was also able to hydrolyze long chain fatty acid esters, as well as triolein. The primary sequence and predicted tridimensional structure of the enzyme are very similar to those of other *Anoxybacillus* and *Geobacillus* carboxyl esterases in a distinct, recently described lipase family. Est/Lip was highly enantioselective, with preference for the (*S*)-enantiomer of substrates.

Keywords: *Anoxybacillus flavithermus*; biochemical characterization; enantioselectivity; gene cloning and expression; thermostable esterase/lipase; tridimensional structure