Organic-solvent stability of elastase strain K overexpessed in an Escherichia-pseudomonas expression system.

ABSTRACT

The structural gene of elastase strain K (elastase from Pseudomonas aeruginosa strain K), namely HindIII1500PstI, was successfully sequenced to contain 1497 bp. The amino acid sequence, deduced from the nucleotide sequence, revealed that the mature elastase consists of 301 amino acids, with a molecular mass of 33.1 kDa, and contains a conserved motif HEXXH, zinc ligands and residues involved in the catalysis of elastase strain K. The structural gene was successfully cloned to a shuttle vector, pUCP19, and transformed into Escherichia coli strains TOP10, KRX, JM109 and Tuner™ pLacI as well as P. aeruginosa strains PA01 (A.T.C.C. 47085) and S5, with detection of significant protein expression. Overexpression was detected from transformants KRX/pUCP19/HindIII1500PstI of E. coli and PA01/pUCP19/HindIII1500PstI of P. aeruginosa, with increases in elastolytic activity to 13.83- and 5.04-fold respectively relative to their controls. In addition, recombinant elastase strain K showed considerable stability towards numerous organic solvents such as methanol, ethanol, acetone, toluene, undecan-1-ol and n-dodecane, which typically pose a detrimental effect on enzymes; our finding provides further information to support the potential application of the enzyme in synthetic industries, particularly peptide synthesis.

Keyword: Elastase; Organic solvent-tolerant protease; Overexpression; Psedomonas aeruginosa strain K; Shuttle vector.