Cloning and overexpression of extracellular elastase from Pseudomonas aeruginosa

ABSTRACT

This study was an attempt to overexpress the extracellular elastase from Pseuodomonas aeruginosa in Escherichia coli and characterize the level of purified enzymes of recombinant bacterium. The gene encoding an elastase natively produced by Pseudomonas aeruginosa was cloned and overexpressed in Escherichia coli using pET-32a system and the resultant recombinant elastase was purified and compared with the native elastase gene. The 1497 bp gene was amplified and subcloned in pET-32a and subsequently transformed into E. coli BL21. The media assay, SDS-PAGE and Western blotting were carried out to analyze the results, and the extracellular enzyme was purified to detect enzyme activity of recombinant E. coli. Nucleotide sequencing of the DNA insert from the clone revealed that the protease activity corresponded to an open reading frame consisting of 1497 bp coding for a 53.69-kDa protein. The clear zones around the recombinant colonies on skim milk agar as well as sharp band on 53-kD size on SDS-PAGE and Western blotting confirm the correct expression of elastase enzyme. Bacterial culture containing pET-32a-lasB showed high enzyme activity around 670g elastase ml-1. The results showed that elastase has potential to be produced industrially and be applied in medicine, food, etc. divisions.

Keyword: Cloning; Elastase; Overexpression; Pseudomonas aeruginosa