Cloning and Expression of the Nattokinase Gene from the Acid-resistant Wild Type Strain Bacillus subtilis natto and Char

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ABSTRACT

The databases of DYU1001 and DYU1002 strains from Dr. Chen and Dr. Wang are bioassayed as Bacillus subtilis with 16 S rDNA. After generalizing the data of nattokinase, we find that nattokinase can be divided into three fragments：pre , pro and mature. Mature is the primary functional domain of nattokinase, but its acid-resistant ability is not good. The fragment “ pre-pro ” protects “ mature ” from hydrolysis by gastric acid with the sacrificial way. The primer pair was designed in compliance with the amino acid sequence of pre-pro-mature nattokinase. The chromosome of Bacillus subtilis DYU1002 was used as a template to synthesize 1146 bp DNA fragment with PCR amplification, then compare the gene fragment with the amino acid sequence of the Bacillus subtilis ’ pre-pro-mature nattokinase. The identities is 100%. The nattokinases are marketed presently most sold in capsules or powders. Their thrombolytic function to against arteriosclerosis is not very effective, because their activity may be destructed through gastric acid. According that Dr. Wang considered the Bacillus subtilis DYU1002 can grow in acidic environment (pH 3.0), we screen the gene of Bacillus subtilis DYU1002 which have the pre-pro-mature nattokinase activity. Then we transformed this gene into E.coli, and expressed the enzymatic activity of nattokinase. The ORF of nattokinase gene is 1146 bp and it translated into a peptide of 382 residues. The molecular weight of the thrombolytic enzyme is 42 kDa. The enzyme was recoveried and measured their thrombolytic activity. We found that the enzyme has the optimal thrombolytic activity in pH 8.0；the activity in the germ body of enzyme can achieve 0.96×103 FU/mg. In the SDS-PAGE, one obvious protein product from 0-30% ammonium sulfate precipitation was found. Its molecular weight is about 42 kDa. The only one secreted protein from E. coli nattokinase recombinant is called exoenzyme. The thrombolytic activity of this secreted nattokinase was 5.66 × 105 FU/mg. We found that the enzyme has the dural thrombolytic activity in pH3.0~ pH 10.0.

Keywords : Bacillus subtilis ; nattokinase ; gene cloning ; enzyme characterization

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