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Cloning of nattokinase gene from *Bacillus subtilis* BB-1 and its *in vitro* translation

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A fibrinolytic enzyme gene was isolated from *Bacillus sutilis* BB-1 by PCR method. Primers for PCR cloning were designed according to pre-identified gene for fibrinolytic enzymes from *B. subtilis*. The primer sequences are 5′-CGG ATC CGT GAG AGG CAA AAA GGT G-3′ and 5′-TGA ATT CTT AAT GTG CTG CTG CTT GTC C-3′ as concensus sequences of the fibrinolytic genes. The PCR product was 1,145 bp and the sequence homology was 99% with nattokinase gene isolated from japanese natto.

The cloned fibrinolytic gene was *in vitro* transcripted and translated by using Genelator™ transcription and translation kit. The *in vitro* expressed protein is a monomeric protein and had fibrinolytic activity. The optimum pH and temperature are 7.0 and 35℃, respectively.

Substrate specificity of the fibrinolytic enzyme was detected in skim milk, gelatin, casein and blood agar plates. These is no any enzyme activity on these substrates. This means that the enzyme may be used for health-care such as thrombosis without any hamful behavour in the blood vessel.