THE EFFICIENT EXPRESSION OF NATTOKINASE IN *ESCHERICHIA COLI* BY SEQUENCE OPTIMIZATION

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Key words: nattokinase, *Escherichia coli*, sequence optimization, synonymous mutations

Abstract: Nattokinase, a serine protease derived from *Bacillus subtilis natto*, holds substantial promise for medical applications due to its potent and safe thrombolytic properties. However, its low yield and diminished activity have constrained its industrial utility as a thrombolytic agent. The 5' end of gene sequence has been recognized as a pivotal factor influencing its expression. To address this, we introduced a 48-bp random sequence at the 5' end of the red fluorescent protein (mCherry) gene and screened for highly fluorescent mutants via flow cytometry. The resulting sequence was then ligated to the 5' end of nattokinase and cloned into the PET28-a(+) plasmid for expression in *E. coli* BL21(DE3). SDS-PAGE electrophoresis demonstrated a marked increase in nattokinase expression compared to the original sequence. At the same time, we introduced synonymous mutations to 15 codons at the 5' end of nattokinase, leading to significant enhancements in the expression of several variants. Both strategies effectively bolstered nattokinase expression in *E.coli*, offering valuable insights for future investigations