신규 분비서열과 folding catalyst를 이용한 대장균에서 재조합 단백질의 효율적 분비생산

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Escherichia coli has been the workhorse for manufacturing recombinant proteins, because it is a well-characterized bacterium with many available expression systems. However, the use of *E. coli* for the production of recombinant proteins has faced many practical and biological problems. *E. coli* cannot be used for the production of some complex, large proteins containing complex disulfide bonds, or mammalian proteins that require post-translational modifications for activity. The stability of foreign proteins produced in *E. coli* is often low due to proteolytic degradation. Proteins overexpressed are often produced in the form of inclusion bodies, from which biologically active proteins can only be recovered by complicated and costly denaturation and refolding processes.

Secretory production of recombinant proteins using signal sequence has several advantages. First, the N-terminal amino acid residue of the secreted product can be identical to the natural gene product as a result of the cleavage of the signal sequence by a specific signal peptidase; Secondly, protease activity is considered to be much less in the periplasmic space than in the cytoplasm; Thirdly, the purification process of recombinant protein is much simpler due to the presence of less contaminating proteins in the periplasm; Fourthly, correct formation of disulfide bonds can be facilitated because periplasmic space provides oxidative environment.

For the efficient secretory production of proteins in *E. coli*, a number of signal sequences including OmpA, OmpF, PhoA, SpA and PelB have been used. However, protein secretion into the periplasm is a complex and incompletely understood process, and therefore, the use of such signal sequences does not always ensure the successful secretion into periplasm of *E. coli* due to the

several reasons as follows. First, the signal sequence is often incompletely processed or removed. Secondly, the amount of recombinant proteins secreted is often low or undetectable. Thirdly, autolytic activities of cells caused by weakened outer membrane are often observed. Fourthly, secretion efficiency varies considerably depending on the characteristics of the proteins to be secreted. Under these circumstances, there are strong reasons for the development of novel signal sequences allowing the efficient secretory production of recombinant proteins.

In this work, we described the high efficient secretion of several recombinant proteins into periplasm of *E. coli* using a novel endoxylanase signal seqeuence from *Bacillus* sp. and artificial signal sequence. Secretory production at high cell density was also examined. We also demonstrated the coexpression of oxidoreductase DsbA of *E. coli* increased the solubility of human leptin significantly in the periplasmic space.

These findings suggest that the *Bacillus* sp. signal sequence and artificial signal sequence can be added to the list of a few available signal sequences useful for the high-level secretory production of various recombinant proteins in *E. coli*.

Acknowledgement

This work was supported by Ministry of Commerce, Industry and Energy (MOCIE) through the Institute of Industrial Technology Policy (ITEP).

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