Effects of periplasmic targeting and culture medium supplementation with glycine and tween-20 on extracellular production of recombinant proteins in Escherichia coli

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Abstract:
Extracellular production of recombinant proteins using Escherichia coli is desirable but recombinant pharmaceutical proteins. However, the lack of a secretion system for efficiently excreting recombinant proteins into the extracellular medium remains a problem to be solved. In this study we designed a recombinant plasmid encoding a periplasmic L-asparaginase as a model protein and evaluated the effects of different concentrations of glycine and tween20 on cell density and protein leakage from periplasmic space into culture medium.

A plasmid containing L-asparaginase in fusion with a periplasmic signal peptide was designed and used for transformation of E. coli BL21. The recombinant cells were cultured in LB broth at 37°C. IPTG as an inducer and different concentrations of glycine and tween-20 were added to culture medium at OD600 of 0.6. Protein analysis was performed by SDS-PAGE after 4 h of induction. The enzyme activity was determined by Nesslerization method. SDS-PAGE analysis revealed a prominent band of 37 kDa corresponding to the predicted size of the target protein. Enzyme activity in culture supernatant was increased up to 782 U/L with 1.5% (v/v) tween-20. Maximum enzyme activity of 1090 U/L was achieved with 0.75% (w/v) glycine, while no asparaginase activity was detected in the culture medium without supplementation.

Our results showed that periplasmic targeting of the recombinant protein in E. coli and culture medium supplementation with glycine and tween-20 increase protein secretion into the extracellular space where direct export of proteins is very difficult to obtain.

Keyword: extracellular, E.coli, glycine, tween-20