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Research Article

Extracellular secretion recombinant of human epidermal growth factor (hEGF) using pectate lyase B (PelB) signal peptide in escherichia coli BL21(DE3)

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ABSTRACT

The extracellular expression of high valued therapeutic proteins such as Human Epidermal Growth Factor (hEGF) from Escherichia coli has become a challenge yet give some advantages. It ensures the correct folding of recombinant hEGF (rhEGF) so the therapeutic activity and immunogenicity profile will be similar with the native hEGF. In addition, extracellular expression eliminates the host cell disruption and simplifies the purification process, although other study has shown the protein yield from E. coli extracellular expression is lower than the intracellular expression. Various factors such as codon usage, inducer concentration, induction time, and harvest time can be optimized to increase the rhEGF secretion. The aim of this research is to express rhEGF extracellularly from E. coli BL21 (DE3). The expression system was supported by optimized codon usage to E. coli codon preference, pectate lyase B (PelB) signal peptide in the pD881-PelB expression vector, and L-rhamnose as an inducer. Growth curve of E. coli was made to determine the L-rhamnose induction time. Induction was performed with 4mM L-Rhamnose at OD600 0.7. rhEGF in the soluble fraction, periplasmic extraction, and culture medium was characterized by tricine SDS-PAGE. Quantification of rhEGF concentration was performed by ELISA. The codon optimization showed that the Codon Adaptive Index (CAI) of rhEGF gene was 1, GC percentage was 50.93% and relative adapts was 100% after codon usage optimization. SDS-PAGE showed 6.2 kDa band of rhEGF band from soluble fraction, periplasmic extraction (after 18 hour induction), and culture medium. The quantification of rhHEGF by ELISA showed the rhEGF concentration was 310.8 µg/mL.

Keywords: Recombinant human EGF; Codon optimization; PelB signal peptide; Extracellular expression of *E. coli* BL21 (DE3); Periplasmic extraction.

INTRODUCTION

Epidermal Growth Factor is a polypeptide containing 53 amino acids and having 3 disulfide bonds. It was firstly discovered on the submaxillary gland of rats in 1962 by Cohen et al.while Human Epidermal Growth Factor (hEGF) was first isolated from urine in 1975 by Starkey et al. (Gainza et al., 2015). hEGF was known as a potent mitogenic factor for maturation on epidermal cell. Many studies showed the ability of hEGF in stimulating the cell proliferation in tissue culture (Dinh et al., 2015). Researchers agree that hEGF is strongly related to the released thrombocyte and growth factor during blood clotting. The experiment on animal test has showed the treatment of EGF on the condition animal was proved to help the increase of wound healing (Zhang et al., 2015).

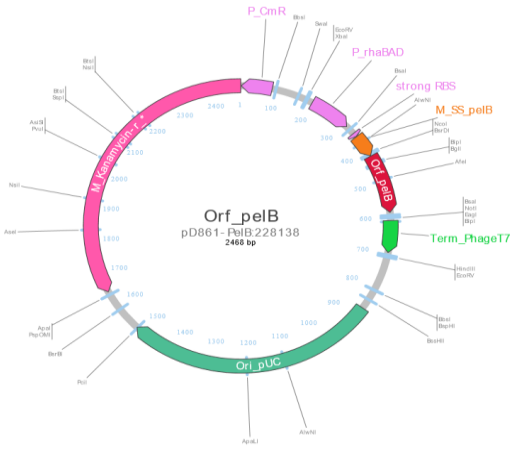

Figure 1: Plasmid map of pD881-PelB

Some studies that have been done since 1975 by Cohen & Capenter showed hEGF gave significant result in healing chronic wounds, corneal injuries, and gastric ulcers (Huang et al., 2001). The increasing needs for hEGF in clinical applications, many studies have attempted to increase the production of recombinant hEGF (rEGF) as terapeutic protein by recombinant DNA

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