**Generation Of Therapeutic And Diagnostic Recombinant Proteins In The Periplasm Of *Escherichia coli* W3110 ΔompC**

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**Background and aims.** With the increasing demand for improved diagnostics for cancer and therapeutics for diabetes, the development of efficient recombinant protein production systems has become essential. Periplasmic protein expression in *Escherichia coli* promotes proper protein folding and simplifies downstream purification. In this study, we utilize the *E. coli* W3110 ΔompC strain to express two medically relevant recombinant proteins: nanobody NTV1 (targeting VEGFR2, a biomarker closely associated with angiogenesis and tumor growth), and proinsulin (a precursor of insulin).

**Methods.** The coding sequences of human proinsulin and NTV1 was cloned into plasmid pSBinit (#Addgene 110100) and subsequently transformed into *E. coli* W3110 ΔompC. The bacteria were cultured in AMS minimal medium and periplasmic proteins were extracted using osmotic shock combined with lysozyme lysis. Proinsulin was purified by ion exchange chromatography, while NTV1 was purified by nickel affinity chromatography. The binding ability of NTV1 to VEGFR2 was analyzed by direct ELISA and IC₅₀ value was used as a measure of interaction strength. For proinsulin, enzymatic digestion with trypsin and carboxypeptidase was performed, resulting in insulin and C-peptide products which were then detected by ELISA to indirectly evaluate the qualitative of proinsulin.

**Results.** The periplasmic proinsulin extracted from E. coli W3110 ΔompC yielded a concentration of 1.75 mg/mL, with a ~9.4 kDa band observed on SDS-PAGE, and enzymatic digestion confirmed the presence of insulin and C-peptide, indicating successful expression and processing of proinsulin. Meanwhile, NTV1 was obtained at 0.389 mg/mL, with a ~15 kDa band observed on SDS-PAGE, and ELISA analysis confirmed its specific binding to VEGFR2, with an IC₅₀ of 4.161 µg/mL (95% CI: 1.861–9.304 µg/mL), thus demonstrating strong affinity.

**Conclusion.** This study successfully generated a recombinant E. coli W3110 ΔompC strain expressing validated proinsulin and NTV1. These findings highlight the potential of utilizing recombinant E. coli W3110 ΔompC strain for production of recombinant proteins, aiming for future diagnostic and therapeutic applications.

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