AN EXTRACELLULAR PROTEIN EXPRESSION SYSTEM IN ESCHERICHIA COLI IMPLIES POTENTIAL APPLICATION

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Key Words: Escherichia coli, extracellular expression, cellulose

Escherichia coli is commonly used as a host for the extracellular production of proteins. However, its secretion capacity is often limited to a frustratingly low level compared with other expression hosts, because *E. coli* has a complex cell envelope with two layers. We recently identified the catalytic domain of a cellulase (Cel-CD) from *Bacillus* sp. that can be secreted into the medium from recombinant *E. coli* in large quantities without its native signal peptide. By subcellular location analysis, we verified that the secretion was a two-step process via the SecB-dependent pathway through the inner membrane and an unknown pathway through the outer membrane. However, the N-terminal region of Cel-CD is polar and hydrophilic, which showed no similarities to other typical signal sequences. Random mutagenesis experiment suggested that the N-terminal sequence is a compromising result of transportation through inner and outer membranes. This is the first report that a "non-classical signal peptide" can guide recombinant proteins out of the cells from cytoplasm. Both the Cel-CD and its N-terminal sequence can serve as carriers for efficient extracellular production of select target proteins with a concentration from 101 to 691 mg/L in flask cultivation.

This protein can degrading cellulose efficiently in the culture medium indicating a great potential. Therefore, a recombinant *E.coli* that can directly utilize cellulose as sole carbon source by fusion Cel-CD with a β-glucosidase was constructed. Recombinant strains were confirmed to use the amorphous cellulose as well as cellobiose as the sole carbon source for growth. Furthermore, both strains were engineered with poly (3-hydroxybutyrate) (PHB) synthesis pathway to demonstrate the production of biodegradable polyesters directly from cellulose materials without exogenously added cellulases. The results suggested that this system has a potential application in lignocellulosic biomass degradation and biochemical biofuel production.
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Reference

1. Exploring the N-terminal role of a heterologous protein in secreting out of Escherichia coli, Biotechnol Bioeng. 2016 Dec;113(12):2561-2567. doi: 10.1002/bit.26028. Epub 2016 Jun 14.
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