

ABSTRACT:

Direct transport of recombinant protein from cytosol to extracellular medium offers great advantages, such as high specific activity and a simple purification step. This work presents an investigation on the potential of an ABC (ATP-binding cassette) transporter system, the hemolysin transport system, for efficient protein secretion in *Escherichia coli* (*E. coli*). A higher secretory production of recombinant cyclodextrin glucanotransferase (CGTase) was achieved by a new plasmid design and subsequently by optimization of culture conditions via central composite design. An improvement of at least fourfold extracellular recombinant CGTase was obtained using the new plasmid design. The optimization process consisted of 20 experiments involving six star points and six replicates at the central point. The predicted optimum culture conditions for maximum recombinant CGTase secretion were found to be 25.76 μ M IPTG, 1.0% (w/v) arabinose and 34.7°C post-induction temperature, with a predicted extracellular CGTase activity of 68.76 U/ml. Validation of the model gave an extracellular CGTase activity of 69.15 ± 0.71 U/ml, resulting in a 3.45-fold increase compared to the initial conditions. This corresponded to an extracellular CGTase yield of about 0.58 mg/l. We showed that a synergistic balance of transported protein and secretory pathway is important for efficient protein transport. In addition, we also demonstrated the first successful removal of the C-terminal secretion signal from the transported fusion protein by thrombin proteolytic cleavage.