Enhanced secretory production of hemolysin-mediated cyclodextrin-glucanotransferase in Escherichia coli by random mutagenesis of the ABC transporter system.

ABSTRACT

The hemolysin transport system was found to mediate the release of cyclodextrin glucanotransferase (CGTase) into the extracellular medium when it was fused to the C-terminal 61 amino acids of HlyA (HlyAs(61)). To produce an improved-secretion variant, the hly components (hlyAs, hlyB and hlyD) were engineered by directed evolution using error-prone PCR. Hly mutants were screened on solid LB-starch plate for halo zone larger than the parent strain. Through screening of about 1 × 10(4) Escherichia coli BL21(DE3) transformants, we succeeded in isolating five mutants that showed a 35-217% increase in the secretion level of CGTase-HlyAs(61) relative to the wild-type strain. The mutation sites of each mutant were located at HlyB, primarily along the transmembrane domain, implying that the corresponding region was important for the improved secretion of the target protein. In this study we describe the finding of novel site(s) of HlyB responsible for enhancing secretion of CGTase in E. coli.

Keyword: Alpha-hemolysin transport system; Cyclodextrin glucanotransferase; Directed evolution; Extracellular secretion; Escherichia coli.