Cyclodextrin glycosyltransferase from *Brevibacillus brevis*: from gene to application

Hwa-Gyun Shin[1,2,3], Jung-Oh Ahn[2], Eun-Gyo Lee[2], Kyung-Jea Kim[3], Hong-Weon Lee[2], Joon-Ki Jung[2,*] Young-Ha Lee[1]

Chung Nam National University[1], *Korea Reserch Institute of Bioscience & Biotechnology(KRIBB)*[2], *Daepyung co., Ltd*[3]

TEL : + 042-860-4513, FAX : + 042-860-4516

Abstract

Production of a cyclodextrin glycosyltransferase (CGTase) from *Brevibacilus brevis* CD162, which converts starch predominantly to β-CD at high conversion yields, was studied. Expression of this gene using pET22b(+) vector in *Escherichia coli* resulted in the formation of inactive inclusion bodies. Under the control of lac promoter in pUC19, it was intracellularly expressed as soluble forms, reaching 11,000 U/ml of CGTase activity in high-cell density fed-batch culture. For the secretory production of CGTase, A expression vector system was developed. The mature CGTase gene was fused to α-amylase signal sequence from *Aseprgillus oryzae* for the effective secretion into the culture broth and expression was controlled under GAL10 (the gene coding UDP-glactose epimerase of *S. cerevisiae*) promoter. *S. cerevisiae* harboring the resulting plasmid successfully secreted CGTase. In addition, when the cellulose-binding domain (CBD) from *Trichoderma harzianum* endoglucanase II (THEG) was connected to the N-terminial of CGTase through an endogenous linker peptide from THEG, secretion of CBD-Linker-CGTase by this fusion construct was dramatically 5-fold enhanced, compared with that of the mature CGTase. The fusion protein was secreted into the culture medium, reaching 14,000 U/mL of CGTase activity in high-cell density fed-batch cultures. For the industrial application of CGTase to glycosylation of stevioside, the genetically modification of CGTase was also studied.

Reference

1. Myung Hee Kim, Cheon Bae Sohn, Tae Kwang Oh, Cloning and sequencing of a cyclodextrin glycosyltransferase gene from *Brevibacillus brevis* CD162 and its expression in *Escherichia coli*(1998), FEMS Microbiology Letters Vol. 164, pp. 411～418.