Research in Pharmaceutical Sciences, 2012;7(5)
School of Pharmacy and Pharmaceutical Sciences
Isfahan University of Medical Sciences

Down-regulation of acetate pathway through antisense RNA against ACKA and PTA in *Escherichia coli*: Improved beta interferon production

M. Morowvat[1,*], V. Babaeipour[2], H. Rajabi Memari[3], H. Vahidi[1]

[1]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Biochemical Engineering Group, Biotechnology Research Center, Malek-Ashtar University of Technology, Tehran, Iran
[3]Center of Biotechnology and Life sciences, Shahid Chamran University of Ahwaz, Ahwaz, Iran

Background and Aims: Escherichia coli is one of the most widely used hosts for the production of recombinant proteins. The accumulation of the metabolic by-product acetate is the major problem of using E. coli as an expression system. Various strategies including genetic or process modification have been developed to limit acetate accumulation or reduce its negative effects to increase the productivity of recombinant proteins. Although these strategies can lead to an improved process performance, their implementation is very difficult in practice. In this study an antisense RNA strategy was employed as an elaborate metabolic engineering tool to partially block biosynthesis of two major acetate pathway enzymes, phosphotransacetylase (PTA) and acetate kinase (ACK).
Methods: Based upon pET-25 b(+), a recombinant plasmid containing antisense genes targeting pta and ackA was designed and its effects on the acetate pathway and foreign protein productivity compared to the control plasmid was determined in E. coli BL21 (DE3). Beta interferon was employed as a model foreign protein. Timing of antisense and beta interferon expression was controlled by using the intrinsic ackA promoter and T7 promoter, respectively.
Results: The mRNA levels of target enzyme genes (ackA and pta) were lowered in transformed cells compared to wild type cells. The concentration of acetate in culture media was decrease using the constructed plasmid. The expression of antisense RNA did not affect the cell growth negatively. The total production of beta interferon was enhanced in antisense-regulated strain compared to control plasmid without any antisense genes.
Conclusions: Application of an antisense strategy on the acetate pathway was successful in metabolically engineering E. coli to improve recombinant protein production. This enhancement of production yield by antisense technology suggests that this strategy may be successfully applied to practical large-scale high cell density fermentations of E. coli expression system.

Keywords: Antisense RNA; *Escherichia coli*; Beta interferon; Acetate production; Metabolic engineering