Mass production of peptides in recombinant *E. coli* by analysis of metabolic load

Abstract

Metabolic load caused by overproduction of recombinant protein inhibits host cell growth and sometimes it becomes the one of the reason of death of host cell. For optimized production of recombinant protein, protein production rate and cell growth rate have to be finely tuned. Guanosine tetraphosphate (ppGpp) is an alarmone of stringent response which occurs under such metabolic load. Metabolic load of host cell can be determined by measuring the intracellular ppGpp concentration. Reverse phase HPLC was used to determine the intracellular ppGpp concentration.

Recombinant *E. coli* producing anti HIV peptide T20 was used as model system. During the fed-batch fermentation, cell growth, peptide production, and ppGpp level were measured. From these data, the occurrence of metabolic load was determined for each wild type cell and peptide overproducing recombinant cell. Then, whole cell proteins of these two cell types were analysed by 2-D gel electrophoresis to determine what is the cause of metabolic load so called stringent response and how can this be overcome. From this analysis, 23 protein expression level were significantly altered. The expression level of transladolase (talB) and acyl-carrier protein transacylase (fabD) were increased more than five times, so these two genes might be the key genes to solve metabolic load. Two low level expression plasmids pACYC184/talB and pACYC184/fabD were constructed and the effect of enhancing these two genes expression was examined.

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