Abstract

Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) is a hematopoietic growth factor involved in the growth and maturation of different cell types. Recombinant GM-CSF, both *E. coli* and yeast derived have been successfully used as a therapeutic agent in facilitating bone marrow and stem cell transplantation and in other clinical cases like neutropenia. Commercial production of rhGM-CSF has faced the problem of low specific activity in many hosts including *E. coli*. In this study human GM-CSF was cloned from peripheral blood cells. This gene (NCBI, accession number: AF510855) was used to transform *E. coli* strains and expression studies were done with various host vector combinations under a wide range of conditions. It was found that the toxicity of rhGM-CSF towards its host was the major reason for its low specific activity. This problem was circumvented in part by growing the cells in enriched medium. Periplasmic transport of rhGM-CSF proved to be the most successful strategy to increase its specific activity.

Expression of rhGM-CSF was also explored in a new host, the methylotrophic yeast *Pichia methanolica*, both in the cytoplasmic and secretory form, the latter showing a higher specific activity. Secretory expression of rhGM-CSF was also done in *Pichia pastoris* using a constitutive and inducible system. High specific activities were obtained with an inducible recombinant strain of *P. pastoris* carrying two copies of the hGM-CSF gene. Bioreactor studies were done to scale up rhGM-CSF production in both *E. coli* and methylotrophic yeast. Finally the different rhGM-CSF molecules generated in this study were assayed for biological activity and were found to be active.