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GENE EXPRESSION AND MOLECULAR STUDIES WITH CORYNEBACTERIAL PLASMIDS

by

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Dedicated
to my Parents

CERTIFICATE

This is to certify that the thesis entitled " **Gene expression and molecular studies with corynebacterial plasmids**" being submitted by **Preeti Srivastava** to the Indian Institute of Technology, Delhi, for the award of Degree of **Doctor of Philosophy**, is a bonafide research work carried out by her under my supervision and guidance. The results presented in this thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.

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ABSTRACT

One of the main goals of recombinant DNA research is to produce significant quantities of medically and agriculturally important proteins whose genes have been cloned on bacterial plasmids. It is possible to express large quantities of virtually any protein in convenient expression hosts. The choice of an expression system for the high-level production of recombinant proteins depends on many factors. These include cell growth characteristics, expression levels, intracellular and extracellular expression, posttranslational modifications and biological activity of the protein of interest, as well as regulatory issues in the production of therapeutic proteins. *Escherichia coli* has been the work-horse for recombinant protein production for more than two decades. However, in spite of the extensive knowledge on the genetics and molecular biology of *E. coli*, not every gene can be expressed efficiently in this organism. This may be due to degradation of the protein by host cell proteases, inclusion body formation inside the cell or potential toxicity due to the release of pyrogens from the cell wall of these bacteria. Non-pathogenic soil corynebacteria are GRAS grade organisms. Two important characteristics of these bacteria that make it ideally suited as an expression system are its non-sporulating nature and low proteolytic activity. Several cryptic plasmids have been isolated and characterized in these bacteria. Using these plasmids cloning vectors, promoter-probe vectors and a few expression vectors have been constructed. The present study is, therefore, aimed at constructing expression and secretion vectors of corynebacteria to increase the available gene expression tools. Plasmid pBL1 from *Brevibacterium lactofermentum* was used in all these studies. Several expression vectors

and cloning vectors were constructed. Expression vectors were made using *E. coli trc* promoter. For ease of manipulation an *E. coli*-corynebacteria shuttle vector was developed. A series of gene-fusion vectors based on glutathione-S-transferase were also constructed. A T7 RNA polymerase and T7 promoter based host vector system was developed in this bacteria for the first time. *E. coli* β-galactosidase and streptokinase were used as reporter genes in all these constructs. The level of expression of streptokinase achieved using T7 RNA polymerase-T7 promoter based system was much higher as compared to that achieved using the gene-fusion system. A secretion vector using diphtheria toxin promoter and signal sequence was also constructed. β-galactosidase, which is a highly fastidious protein not amenable to secretion was largely secreted out when expressed in *B. lactofermentum* using this vector. Expression in the secretion vector is regulated by iron. Gene for enhanced green fluorescence was also cloned in the gene-fusion vector, which suggested that even eukaryotic signals are recognized efficiently by these bacteria so far as this protein is concerned. Molecular studies were carried out with small *C. renale* plasmids to assess their potential for use as vectors. Since understanding of basic plasmid molecular biology would be advantageous in determining the usefulness of a plasmid vector, investigations were carried out on plasmid incompatibility, host range, stability, copy number and mode of replication of the two smaller plasmids pCR1 and pCR2. The smallest plasmid pCR1 replicates by rolling circle mechanism. This plasmid and pCR2 require DNA Polymerase I for their replication in *E. coli*. Plasmid pCR1 can replicate in *E. coli* and several species of corynebacteria. It has a copy number 1.4 times that of pUC19. It is incompatible with corynebacterial plasmid pBK2 and *E. coli* plasmids pUC19 and pACYC184. This plasmid is non-conjugative but mobilizable.

It has an *oriT*, which shows homology to the left border and right border sequence of Ti plasmid of Agrobacterium. Plasmid pCR2 has a very broad host range. It can replicate in *E. coli*, Citrobacter, Pseudomonas, *B. subtilis*, *M. smegmatis* and several species of corynebacteria. It is compatible with corynebacterial plasmid pBK2 and *E. coli* plasmid pUC19 and pACYC184. The minimum replicon of this plasmid was determined and it was found to lie in a 1.4 kb region. Besides, these plasmids do not adversely affect the host physiology. Thus, these plasmids have tremendous potential for the development of versatile vectors.

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