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17194

February 1988

BIOSCIENCE AND ENGINEERING

DP/IND/80/003

Republic of India

Report on: Consultant in Biotechnology

Prepared for the Government of India

by the United Nations Industrial Development Organization

acting as executing agency for the United Nations Development Programme

Based on the work of Prof. A. Therwath

Consultant in Biotechnology

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REPORT OF MISSION

I spent 15 days, starting 1st December 1987, as the UNIDO consultant at the Biochemical Sciences Division of the National Chemical Laboratory (N.C.L.) in Pune, India. My mission to NCL was to organize a Biotechnology research setup and initiate technology of recombinant DNA.

The objective of the group in Pune is to mass produce at low cost, through the technique of genetic engineering and cloning, industrially important macromolecules. This is an alternative to the lengthy and laborious classical chemical production procedure.

Molecular cloning of the Xylanase/cellular Genes from Streptomyces

Hemicellulose and cellulose are the most abundant organic compounds in nature and form the major constituents of plant biomass. The efficient conversion of this otherwise cheap and largely abundant biomass to utilizable products such as Glucose, Xylose, Ethanol and finally to Ethylene involves the combined action of enzymes such as cellulases and hemicellulases. The separation in pure form the enzyme Xylanase free from the contaminating cellulases has an additional and important application in Paper and Pulp industry for improving the quality of paper. These two groups of enzymes have been extensively studied at the NCL with respect to their properties, production and use in hydrolysis of lignocellulosic substrates.

In the course of screening for novel actinomycetes and fungi several thermophilic and alkalophilic actinomyces and Bacillus strains secreting the enzyme Xylanase with interesting properties and potential for exploitation at the industrial level have been isolated at the NCL. So far these efforts were within the framework of fundamental research and therefore remained of academic interest. However with this valuable information already gathered, we decided to apply the techniques of gene manipulation to one of the Xylanase enzyme producing Streptomyces strains. In very simple terms we planned the following experiments :

A) Excise out from the total genome of the microorganism, streptomyces, and from amidst several throusands of different genes, exclusively the gene sequence corresponding to the coding information of the enzyme Xylanase.

B) Take this unique gene sequence out and artificially fuse it into the plasmid DNA of an expression Vector capable of high rate self replication producing within a matter of few hours several thousand identical copies of the fused and recombined gene.

C) Transfer the genetically engineered plasmid into the simple and harmless Eicherichia Coli bacteria and provoke the fused and recombined gene-plasmid to transcribe specific messenger RNAs and to express distinct and functional proteins in E. Coli.

Large volumes of cultures of E. Coli harboring the recombinant plasmid and actively producing the enzyme Xylanase can be grown inexpensively with a minimum laboratory facility.

D) Isolate from lysates of such cultures and through simple and routine procedure the Xylanase enzyme.

Starting day 1 (1st Dec) a small group of scientists composed of two senior investigators and two juniors were birefed in detail regarding the project and detailed discussions covering all major aspects were held. Experimental protocol was worked out the following day and elaborated in detail taking into consideration local conditions and facilities available.

Thus one complete excercise on the cloning of streptomyces DNA into E. Coli expression vector pUC8 was carried out under my supervision.

This involved carrying out successfully the foloowing experiments :

1. Isolation of high molecular weight DNA from Streptomyces.
2. Isolation and purification of the multi-copy expression vector pUC8.

3. Restriction endonuclease digestion of the vector and the Streptomyces genomic DNA followed by enzymatic dephosphorylation of their 5' termini.
4. Ligation of the vector DNA to enzymatically cleaved fragments of the genomic DNA, using bacteriophage T4 DNA ligase. Three sets of experiments in different combinations were performed :

a) Dephosphorylated vector DNA to non dephosphorylated genomic DNA fragments.
b) Dephosphorylated genomic DNA fragments to non phosphorylated vector DNA arms.
c) non phosphoryled genomic DNA fragments to non phosphorylated vecto·· DNA arms.

A suitable E. Coli strain was chosen i.e cells of the strain JM 103 and these were stransformed using the above three ligation-DNA mixtures.
5. A limited number of transformant E. Coli colonies containing genetically engineered Streptomyces DNA sequence were obtained.

Detailed analysis of the transformants for the presuce of plasmid DNA was under progress at the moment I was leaving the NCL Laboratory, Pune. I must mention that at this phase, parts of the experiments that required the presence of an expert from outside was carried out with satisfaction. The research group handling this project will pursue further this work and will maintain regular contacts with me to appraise me of the progress and discuss solutions to problems that may arise.