MICROBIAL MASS PRODUCTION

Introduction

Gene manipulation technology is the most important tool considered as the back bone of modern biotechnology. Presently diverse techniques are involved in the production of insulin, growth hormone and monoclonal antibodies. These are the modern medicines produced by the genetically engineered organisms (FDA approved GRAS –generally regarded as safe organisms). Production of human insulin by recombinant E. coli is considered as a significant outcome of recombinant DNA technology, more complex proteins of medical uses can also be produced by metabolic and cellular engineering of microorganisms. But production of proteins and other derivatives in its native, functional and intrinsic condition is the ultimate challenge of recombinant technology.

Production of Insulin:

Insulin is a peptide hormone mainly used in treatment of diabetes mellitus to control elevated blood glucose level. Banting and Best named it originally as 'isletin' and was later renamed as insulin by Macleod, a word that had been suggested in 1910. This hormone is secreted by the β-cells of the pancreas and consists of two polypeptide chains, A and B which are linked by two inter-chain and one intra-chain disulphide bridge. Insulin is synthesized as a single-chain precursor, pro-insulin, and produced by the proteolytic processing of pro-insulin in the pancreas (Kjeldsen et al 1999). Originally insulin was first identified from dog pancreas which was commercially produced from various sources like foetal calf pancreas obtained from slaughter houses. Now human insulin protein is mass-produced through geneticengineering processes. Recombinant DNA technology has been a great enabler in producing human insulin outside the body for being used as a therapeutic. Insulin is the first human hormone produced in bacteria to be tested in humans for medical purposes.

There are many methods for the production of recombinant human insulin in both bacteria and yeast. One typical scheme for preparing human insulin utilizes pro-insulin that is produced in E. coli cytoplasm as an inclusion body of a fusion protein (Chang 1998). Manufacturing of insulin using microbes as a cell factory involves the following steps – 1. Isolation of gene: The gene for producing human insulin protein is isolated. 2. Preparation of target DNA: Circular piece of DNA called plasmid is obtained from bacteria. 3. Insertion of DNA into plasmid: The gene for insulin is inserted into the plasmid construct. The human insulin gene is now recombined with bacterial DNA. 4. Plasmid insertion: The bacterial DNA having insulin gene is inserted back into bacteria. 5. Plasmid multiplication: The bacterial cells having insulin gene are allowed to grow and multiply and during this process bacterial cells start to produce recombinant insulin. During division newly synthesized copy of cell are produced. 6. Human insulin produced by bacteria is purified.

Production of Growth Hormones:

Growth hormone is one of the most important hormones in human body. The core center for production of growth hormone is pituitary gland. The action of growth hormone is either direct or indirect on the human physiological process. But in some children, malfunction of growth hormone results in abnormal growth of the individual. In case of these conditions recombinant growth hormone is useful for the treatment. Human growth hormone has versatile functions:

• Activates the production of protein in cells by releasing some essential factors.

• Helps in fastening the production of DNA and RNA.

• Accelerates the generation of red blood cells and augments the flow of blood to the kidneys and the rate at which the kidney does its vital filtration work.

• Plays a major role in maintaining the level of fats in the body.

• Activates bone growth and skeletal development indirectly by producing intermediate factor IGF-1.

In order to provide large quantities of IGF-I for physiological investigation and clinical trial, rDNA technology has become the method of choice since large amount of exogenous proteins could be expressed in bacteria. The prokaryote E. coli is preferred as host because of its ease of handling and cultivation, and high yields for many recombinant proteins. Several literature references exist on the production of IGF-I in bacteria as a secreted form fused to secretion leader sequences. (Kim et al 1996).

Lately, biologically and highly active recombinant human insulin-like growth factor-I (rhIGF-I) was produced in yeast (S. cerevisiae). rhIGF-I is a 7.5kDa protein containing 70 amino acid residues, which stimulates the proliferation of a wide range of cell types including muscle, bone and cartilage tissue. IGFs control the biosynthesis of many intracellular and extracellular components and are potent mitogens for MDCs (mesenchymally derived cells). Growth hormone has been shown to mediate its effects on bone formation indirectly, through IGF-I. rhIGF-I is also known as Somatomedin-C is effective and its use apparently involved no special hazards. Recombinant DNA technology is mainly used as a key for production of the growth hormone. In 1979, Dr. Baxter's team and a group of scientists at Genentech succeeded in producing human growth hormone in genetically modified bacteria. Recently, these artificially derived biosynthetic growth hormones are widely exploited in humans.

Production of Monoclonal Antibodies (mAb)

Using Microorganisms as Cell Factories: Monoclonal antibodies are specific antibodies which bind to the particular site of proteins i.e. epitope. Production of the monoclonal antibodies was done from the identical immune cells. They had a major role in treatment of cancer due to their

site specificity. Major techniques involved in the production of recombinant monoclonal antibodies were repertoire cloning or phage display/yeast display. Recombinant antibody techniques use viruses and yeast as a cell factories for the production of monoclonal antibodies.

1) Phage antibody libraries are a variant of the phage antigen libraries first invented by George Pieczenik.

2) All the recombinant antibody techniques rely on cloning the specific gene segments to create the libraries of antibodies. These libraries are specifically different in only few amino acids which decide their specificity.

3) These techniques are applied to improve the specificity with which antibodies recognize antigens, their stability in various environmental conditions, their therapeutic efficacy, and their detectability in diagnostic applications. Monoclonal antibodies production from the fungal species is preferred over the E. coli as: a) Antibodies produced from the fungal systems are extracellular which make protein separation process easier. However recovery of antibodies from the E. coli is very intricate due to its periplasm. b) Common fungal organism exploited for the production of monoclonal antibodies with single chain is the Aspergillus niger. c) Ward et al. expressed full-length IgGs in A. niger using an N-terminal fusion to glucoamylase for both light and heavy chains. They relied on the endogenous KexB protease in A. niger to cleave off the fusion during the secretion of the antibody. (Ward M et al, 2004) d) Monomeric scFv production in P. pastoris was also demonstrated and optimized. (Cunha et al, 2004)

Improvements in the microbial production of antibodies and fragments have resulted from host-cell engineering to give increased and optimized productivity. There is also a trend of producing antibodies or fragments with increased circulating half-life.

Applications of recombinant Monoclonal Antibodies:

a) Diagnostic Applications:

Monoclonal antibodies can be used as a specific probe for developing biosensors and microarray systems.

b) Therapeutic Applications:

• Transplant rejection can be detected immediately with the MAB and CD marker conjugates.

• Most common monoclonal antibodies like Abciximab, Cetuximab are widely prescribed for the cardiovascular diseases and cancer respectively. For breast cancer treatment MAB like Herceptin is a breakthrough invention.

• For treatment of infectious diseases Palivizumab and Briakinumab are widely used whereas for the inflammatory diseases Infliximab are commonly used.

c) Future applications: By exploiting monoclonal antibodies we can combat against the terrorists who can cause threat using biological organisms (Bioterrorism).