Semester – IV
MCB 402: Advanced Microbiology
Gr. A: Natural Therapeutics
Title : Recombinant Therapeutic Proteins

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RECOMBINANT THERAPEUTIC PROTEINS

Many microorganisms represent attractive potential production systems for therapeutic proteins. They can usually be cultured in large quantities, inexpensively and in a short time, by standard methods of fermentation. Production facilities can be constructed in any world region, and the scale of production can be varied as required. The expression of recombinant proteins in cells in which they do not naturally occur is termed 'heterologous protein production'.

Expression systems that are potentially be used for the production of recombinant biopharmaceutical products

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E. coli (and additional prokaryotic systems, e.g. Bacilli)

Yeast (particularly _Saccharomyces cerevisiae_)

Fungi (particular _Aspergillus_)

Animal cell culture (particularly Chinese hamster ovary, CHO; baby hamster ovary, BHK)

Transgenic animals (focus thus far is upon sheep and goats)

Plant based expression systems (various)

Insect cell culture systems

E. coli as a source

It is the most common microbial species used to produce heterologous proteins of therapeutic interest. The first biopharmaceutical produced by genetic engineering to gain marketing approval (in 1982) was recombinant human insulin (trade name Hunulin), produced in *E. coli*.

The **advantages** of *E. coli* as expression system are –

- The molecular biology is well characterized.
- Proteins are synthesized in the inclusion body
- The use of high-expression promoters can increase 30% of total cellular proteins.
- It grow rapidly on relatively simple and inexpensive media, and the appropriate fermentation technology is well established.

E. coli displays a number of **drawbacks** as biopharmaceutical producer

- Heterologous proteins accumulate intracellularly.
- Downstream processing for protein purification is costly.
- Inability to undertake post-translation modification (particularly glycosylation).
- The presence of lipopolysaccharide on its surface.

Some currently marketed therapeutic engineered proteins produced by *E. coli*

Protein	Structure	Trade name	Application
Human insulin	Two chains (A-21Aa; B- 30Aa)	Humulin; Humalog; Novolin	Treatment of diabetes mellitus
Human somatotropin	191 Aa	Protropin; Genotropin; Humatrope; Nutropin; Biotropin	Treatment of human growth hormone deficiency in children
Interferon α_{2a} & α_{2b}	166 Aa	Roferon A; Actimmune	Treatment of various cancers and viral diseases
Interferon γ1b	143 Aa-glycosilated	Actimmune	Treatment of chronic granulomatous disease
Tissue plasminogen activator	530 Aa – glycosilated	Activase	Treatment of acute myocardial infarct and pulmonary embolism
Interleukin 2	133 Aa	Proleukin	Treatment of kidney carcinoma and matastatic melanoma

Yeast : Additional production system

Yeast cells (particularly *Saccharomyces cerevisiae*) display a number of characteristics that make them attractive as production system.

The major advantages are –

- ❄ **Their molecular biolog has been studied in detail, facilitating their genetic manipulation.**
- ❄ **Most are GRAS (generally regarded as safe) – listed organisms, and have a long history of industrial application.**
- ❄ **They grow relatively quickly in relatively inexpensive media**
- ❄ **Suitable industrial scale fermentation equipment / technology is already available.**
- ❄ **They possess the ability to carry out post-translation modification of proteins.**

The major disadvantages are –

- ➢ **The glycosylation pattern usually varies from the pattern of native glycoprotein.**
- ➢ **The level of expression protein is very low, less than 5% of total cellular protein**

Use of recombinant therapeutic proteins of *Saccharomyces cerevisiae*

Proteins	Trade name	Use
Engineered short acting insulin	Novolog	Diabetes mellitus
Colony stimulating factor (GM-CSF)	Leukine	Bone marrow transplantation
All vaccine preparations containing rHBsAg as one component		Vaccination
Hirudin	Revasc, Refludan	Anticoagulant
Urate oxidase	Fasturtec	Hyperuricaemia
Platelet-derived growth factor	Regranex	Diabetic ulcers
Human serum albumin	Albutein	Treatment of hypololemic shock Adjunct in haemodialysis
Hepatitis b surface antigen	Engerix B;HB-Vax II	vaccination

Fungal production systems

Major advantages are –

- Long history of use in the production of various industrial enzyme, antibiotics, etc., therefore, suitable fermentation technology is already exists. They can easily grow in solid media, therefore, production cost is very cheap.
- In general, fungi are capable of high-level expression of various proteins, many of which they secrete into extracellular media.
- The extracellular production of a biopharmaceutical would be distinctly advantageous in terms of subsequent downstream processing.
- They are generally non-pathogenic.
- They have great ability for post translation modification.

Major disadvantages are –

- Most fungus naturally produces extracellular protease, which can potentially degrade the recombinant proteins. This difficulty can be partially overcome by using mutant fungal strains secreting greatly reduced levels of proteases.
- Glycosylation pattern of protein is quite different from the origin of animal cell culture.
- No such biopharmaceutical for such means sought or gained marketing approval.