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**Applications or Uses of Medical Biotechnology**

1. **Genetically Engineered Micro-organisms (GEMOs)**

* The genetically manipulated bacteria and yeasts are collectively called **Genetically Engineered Micro-organisms (GEMOs)..**
* Many GEMOs are employed in biotechnology based industries to manufacture human health care products, biofertilizers and single cell proteins

1. **Health Care Products: (Medical Biotechnology)/ Human health care by the applications of rDNA technology**

Genetically engineered bacteria yield the following health care products:

1. **Insulin:**

* Insulin is a hormone secreted by the beta cells of the islets of langerhans of pancreas
* The deficiency of insulin leads to diabetics in man
* Diabetics is treated by injecting insulin
* This treatment is called **Insulin therapy**
* Eli Lilly (USA) manufactures human insulin in the name of ***Humulin***
* Gilbert et al., made *E.coli* to produce rat insulin to treat diabetics
* Human insulin is also produced in genetically engineered yeasts

1. **Glucagon:**

Glucagon is a hormone secreted by alpha cells of the Islets of Langerhans of Pancrease

It is a h+yperglycemicfctor

It stimulate the breakdown of glycogen and the release of glucose by the liver

In 1998, Eli Lilly manufactured rDNA based glucagon from genetically engineered E.coli cells and marketed in the name Glucagon

It is injected as Insulin for diabetes

1. **Human Growth Hormone:(Somatotropin)**

* Human Growth Hormone (HGH) I secreted by the anterior lobe of the pituitary gland
* It is also called **Somatotropin**
* The deficiency of somatotropin leads to dwarfism in man
* The cDNA of somatotropin was introduced into *E.coli* through the plasmid pBR322
* The genetically engineered *E.coli* produces humangrowth hormone
* It is now sold by Genentech and Kabivitrum
* The human growth hormone increases the height, it is named Somatotropin
* This hormone injecting into the human body increases the height of about 6 cm per year
* It also reduces the ageing process for improving the quality of life

1. **Somatostatin**

* It is a polypeptide hormone secreted by the hypothalamus
* It inhibits the secretion of insulin and glucagon
* Recombinant somatostatin is available in the trade names somatostatin and Stilamin
* It I used for the treatment of bleed gastrointestinal ulcers, deficiency of growth hormones

1. **Interferons:**

* Interferon is an antiviral protein
* It is produced by infected animal cells and it protects the cells from the second viral infection
* Interferons are used in the treatment of common cold, influenza, hepatitis, etc.,
* They induce the production of cellular protein kinase and phosphodiesterasenwyhi9chselectively destroy viral RNAs and proteins
* So the virus fails to multiply in the cells
* The cDNAs of various interferons were isolated from human cell lines and introduced into *E.coli*

The genetically engineered *E.coli* cultures produce interferons such as 1FN-alpha, IFN-Beta, LeIF-D, IFN-alpha1, etc.,

1. **Interleukins**

* Interleukins are small glycoproteins that stimulate the proliferation of Beta cells or T-cells
* They are produced by immunized lymphocytes and are acting as cell-to-cell mediators
* Interleukins behave as a helper fators for enhancing the activity of helper cells and cytotoxic T-cells
* IL-1, IL-2, IL-3 are some examples
* Interleukin – 2 is a single polypepetide with the molecularweight of 30,000 daltons
* It is roduced by T-cells and acting on the activated T-cells
* It stimulates the proliferation of T-cells and hence it is known as T-0-cell Growth factor
* It is used to treat malignant melanoma

1. **Colony stimulating Factors (CSF)**

* Colony stimulating factors 9CSF) are small proteins that stimulate the proliferation of certain immune cells
* Ex: Gp-CSF (Granulocytes-colony stimulating factos stimulates the production of granulocytes in the bone marrow
* The granulocyes in the blood ingest bacteria and other infective particles entered the blood

1. **TNF – Tissue Necrosis Factor or Tumour necrosis factor**

* Tissue Necrosis Factor or Tumour necrosis factor is a protein that slows down the growth and kills cancer cells
* It is 3,00,000 daltons (Da) in molecular weight
* It is an endogenous cytokine produced by macrophages or monocytes when they are exposed to external stimuli
* Once produced, TNF gets accumulated in the cell membrane and causes damage to abnormal cells and cancer cells
* It is used as an anti-cancer drug

1. **Cytokines**
   * + - Cytokines are small proteins that stimulate the migration of cells to the source of cytokines
       - They attract invading cells and destroy them.
       - Ex: TNF Antigen and Tissue Necrosis factor
       - Some cytokines stimulate the immune system
       - For example, the macrophage colony stimulating factor stimulates the production of macrophages in the blood
2. **Blood products:**

* Genetic engineering is used to produce certain proteins that ct on blood or blood producing cells
* Such proteins are often called **blood products**
* They may act as **thrombolytics or blood clotting agents or erythropoietin**
* tPA Tissue Plasminogen are thrombolytics
* They dissolve blood clots and hence they are used **to treat heart attack**
* Factor VIII is a blood clotting agent used to treat haemophilia
* Erythropoietin stimulates the blood production
* Hence it is used to treat anaemia
* The blood products are divided into three categories such as

i. Thrombolytics, ii. Blood clotting factors and iii. Erythropoietin

**i. Thrombolytics**

* Thrombolytics are the compounds that dissolve blood clots in the blood vessels and cavity of the heart
* As they dissolve blood clots, the organs start to function as usual and hence they are known as Bioactive compounds.
* Ex: a. **Tissue Plasminogen activator**

1. **Tissue Plasminogen activator**

* It dissolves blood clots at particular sites without causing any bleeding
* Hence it is used in the treatment of heart attack
* It maintains a balance between thrombolysis and thrombogenesis
* ThuistPA consists of a single polypeptide chain made up of 527 amino acids
* The molecular weight is 65,000 dalton
* It is naturally produced by endothelial cells but in small amounts
* At present, gene manipulation technique has been followed tom produce recombinant tPA (rtPA)
* The (rtPA) is very similar to natural tPA found in the blood stream
* It was produced by Genentech in 1980
* The gene coding for tPA is isolated from human genome which is inserted into a *E.coli* plasmid vector using a proper restriction enzyme and DNA ligase
* As a result, rDNA is obtained which is introduced into *E.coli* cells by transformation using calcium chloride
* The recombinant *E.coli* cells are selected and screened for rDNA
* The recombinant *E.coli* cells are grown in an immobilized cell bioreactor for the production of rtPA on commercial scale
* From the bacterial cultures, rtPA is isolated and purified by centrifugation and electrophoresis
* It is released in the market with the trade name **activase**

**Uses:**

* rtPA cures acute myocardial infarction (Heart attack)
* Lower doses of rtPA reduce the risks of bleeding from soft tissues and veins
* rtPA is administered to dissolve blood clots in deep tissue due to injury

**B. Streptokinase**

* Streptokinase is a thrombolytic enzyme manufactured from colonies of *Streptomyces* bacteria
* It dissolves blood clots in blood vessels and cavity of heart
* Hence it is used for treating heart attacks
* It is also manufactured from genetically engineered *E.coli*

**C. Urokinase**

* It is a thrombolytic enzyme
* It is obtained from human urine or cell cultures of human kidney tissue
* It dissolves blood clots
* So it is used in the treatment of heart attacks
* Now it is manufactured from genetically engineered *E.coli*

**ii. Blood clotting factors**

* These are small proteins that stop bleeding
* They are used to treat haemophilia
* The Armour Pharmaceuticals isolated a blood clotting protein called Factor VIII from human blood to treat haemophilia A
* When the fatr VIII is injected, the blood will clot raidly at wounds
* Hence this fctor is known as anti-haemophilic factor
* **Recombinant factor VIII and factor IX** were isolated from man
* These are used to treat haemophilia

1. **Factor VII**

Factor VII is a trace protein in the blood

It takes part in the coagulation of blood at the site of injury o blood vessels

Factor VII is activated by tissue thrombopolastin

Deficiency of factor VII causes a type of bleeding disorder

It may be inherited opr acquired

It is a rare bleeding disorder in man

One among 500 people may suffer from this disease

This disease is believed to be a type of **haemophilia**

**FactorVII deficiency disorder** is cured by injecting Fresh frozen plasma etc.,

Recombinant factor VII is produced by

* 1. **cell culture technique**, (the gene for factor VII is isolated from human cells and introduced into a mousecell line through virus vector. The recombinant cell is selected and screened for positive production of factor VII)
  2. **transgenic sheep** (External fertilization of egg and sperm of sheep is carried over. The factor VII gene construct is microinjected into the male pronucleus of the fertilized eggs which is then implanted in the uterus of a surrogate mother (sheep)
  3. **Tilapia (fish)** (Factor VII gene of man is microinjected into the eggs of Tilapia. They produce transgenic fingerlings which are grown into mature transgenic fishes. Recombinant factor VII is isolated from the fluid and concentrated to release in the market. Recombinant factor VII concentrate is used to prevent excess bleeding at the time so surgery, to prevent bleeding after child birth in women, to prevent bleeding from joints

iii. **Erythropoietin (EPO)**

* + - * It is a small protein that stimulates the bone marrow to produce more RBC.
      * It boosts the production of RBC to keep it in normal level

1. **Growth factors:**

Growth factors are small protein molecules that stimulate or inhibit cell proliferation

They are produced in tiny amounts in the body

Genetically engineered bacteria have been developed to produce growth factors for human use

Ex: Somatostatin,(for normal functioning of body) neurotropin-3, fibroplast growth factor,

**stem cell factor (SCF)** (stem cell factor or skeletal growth factor stimulates the growth of stem cells in the bone marrow

It increases the growth of bone cells and increases the blood level etc.)

1. **Other Bioactive peptides:**

Bioactive peptides include small proteins which change certain physiological functions of the body

They can be produced from genetically engineered bacteria

Ex: Relaxin – to facilitate childbirth

1. **Vaccines:**

* Genetic engineering is used to develop vaccines against some severe disease such as hepatitis, Foot and mouth disease, AIDS, etc.,
* Recombinant bacteria produce Multiple antigen peptides (MAPs)
* The MAPs are used for vaccination against more than one disease
* Vaccine is a suspension of killed or modified live virus or bacterium being injected into the body to stimulate immunity against the pathogen
* The process of injecting a vaccine in to the body is called as vaccination
* Vaccinated body stars to produce antibodies against the antigen (virus or bacterium) present in the vaccine
* The antibodies act as a defense system and give protection to the body against the particular pathogen
* This type of immunity is called **Acquired immunity**
* Vaccines are used to prevent certain infectious diseases
* Ex: Vaccines for hepatitis, measles, polio, tuberculosis, malaria, leprosy, rheumatic fever, etc.,
* Genetically engineered vaccine contains only a part of pathogen that stimulates the immunity
* It contains one or a few antigens of the pathogen
* As it is a subunit of the pathogen, it is known as subunit vaccine which are also called **biopharmaceutical vaccines**
  1. Recombinant **Hepatitis B vaccine**
     + - Hepatitis B vaccine obtained byusing rDNA techniques is called recombinant Hepatitis B vaccine
       - It is made by cloning a gene for HBs Ag in ***Saccharomyces cerevisiae (yeast)***
       - This recombinant yeast p0roduces 22 nm particles in the cells
       - Such particles alsocan be obtained from *E.coli* also
       - These particles are isolated and purified by ultracentrifugation
       - This vaccine is antigenic, free from contaminants and has no side effect
       - This vaccine is given in three doses as the plasma derived vaccine ha been given
       - It is given at 0,1” and 6” months by intramuscular injection
       - It is available in the trade names Engerix-B, Recomnivax-B, etc.,
  2. **FMD vaccine** (**F**oot and **M**outh **v**accine)
* The vaccine used to immunize cattle against foot and moth disease is called foot and mouth disease vaccine
* It is a viral disease
* It is caused by foot and moth disease virus
* There is a great loss of cattle I India due to this disease
* Recombinant FMD vaccine
* The recombinant vaccine virus with immunogenic FMD virus is used to immunize cattle against FMD.
* FMD was eradicated from Europe by using this recombinant subunit vaccine

**How to produce recombinant FMD vaccine?**

1. The gene producing for immunogenic protein of foot and moth disease virus is identified and isolated from that virus
2. DNA of a ***vaccinia virus*** is isolated
3. The viral DNA is cut with a restriction enzyme to generate linear DNA
4. The gene for the immunogenic protein of FMD is inserted into the linear viral DNA using DNA ligase
5. As a result, an rDNA is formed which is then mixed with coat protein of vaccine virus
6. As a result, mature recombinant ***vaccinia virus*** particles are formed
7. The recombinant vaccinia virus expresses the immunogenic protein of FMD virus in the cytoplasm of mammalian cells
8. The recombinant vaccinia virus is allowed to infect a mammalian cell-line in a culture
9. This virus multiplies in the mammalian cells and forma large quantity of recombinant vaccinia virus
10. Then the recombinant vaccinia virus is isolate from the cell lysate and freeze dried for about 48 hours
11. Freeze drying attenuates the recombinant vaccinia virus and makes it suitable for vaccination

**DNA vaccines for Rabies**

* In recent years, DNAS coding for antigens of pathogens are directly injected into animals to stimulateimmunity. Such DNAs are called DNA vaccines
* The rDNA thus constructed is injected into the host to vaccinate the individuals
* The injected DNA transcribes and translates into the antigen which stimulates the immune system to produce immunity
* DNA vaccine was first discovered by Wolf in 1990
* When injected a necked DNA into mice, the DNA expressed the encoded protein in the mice
* The exogenous DNA, if codes for an antigen, could produce antigenic protein in animals
* This is the principle of DNA vaccines
* The DNA vaccines are often known as third vaccines which are in the pre-clinical stage of vaccine development
* DNA vaccines have been developed for Rabies, Japanese

Encephalitis virus, Malaria, etc.,

* The efficiency of DNA vaccines is hoped that they can be used against serious diseases in man also

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