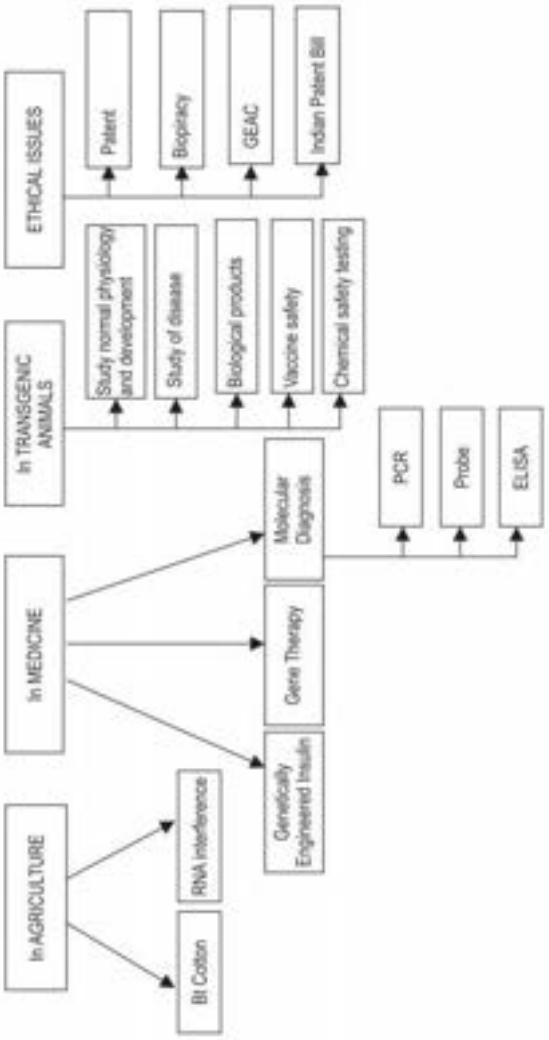
Chapter - 10

Biotechnology and its Applications



Biopesticides : Biological agents that are used to control weeds, insects and other pests.

***cry* Gene :** The Bt toxins are coded by a gene named cry.

***Cry* Protein :** The insecticidal protein which is produced by *Bacillus thuringiensis.*

Green Revolution : Substantial increase in crop yields due to use of high yielding varieties, use of fertilisers and pesticides, improved agricultural practices etc.

Genetically Modified Organisms (GMO) : The organisms which have altered genes in them. These are also known as transgenic organisms.

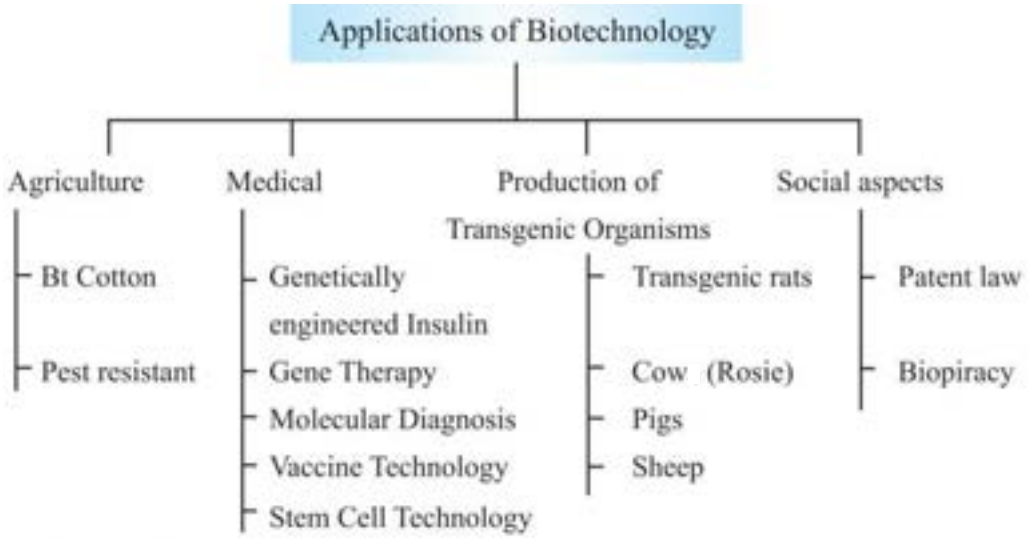
e.g. Bt Cotton, Bt Corn, Transgenic rat, Transgenic cow-Rosie

Molecular Diagnosis : Refers to early detection of diseases using recombinant DNA molecules and techniques like PCR and autoradiography.

RNA Interference (RNAi) : Process used to develop pest resistant plants. It involves silencing of a specific mRNA due to complementary double stranded RNA.

Sustainable Agriculture : It involves organic farming and other integrated management practices which maintain soil fertility while increasing crop productivity.

Use of GM Plants : Tolerant to abiotic stress, Reduced dependence on chemical pesticides, less post harvest-loss, Efficient use of minerals, enhanced nutritional value.



Bt. Cotton : The soil bacterium *Bacillus thuringiensis* produced crystal protein called cry protein that kills certain insects larvae such as tobacco budworm, armyworm, beetles and flies.

- Bt, toxin protein exists as inactive pro-toxins, but once an insect ingest this inactive toxin, it is converted into active form of toxin due to the alkaline pH of the gut which solubilize the crystal. This causes swelling and lysis of epithelial cells of midgut leading to death of insect larvae.
- Bt toxin genes were isolated form *Bacillus thuringiensis* and incorporated into the several crop plants such as cotton.
- The proteins encoded by the genes : crylAc and cryllAb control the cotton bollworms and crylAb controls corn borer.

Pest Resistant Plants : A nematode *Meloidegyne incognitia* infects tobacco plants and reduces their yield.

- Nematode specific genes were introduced into the host plant using *Agrobacterium* as a vector.
- The introduction of DNA was such that it produced both sense and antisense RNA in the host cells.
- These two RNAs being complementary to each other formed a double stranded RNA (dsRNA) making it inactive.

- The nucleotide formed by the process called RNA interference (RNA i).
- The result was that the parasite could not survive in the transgenic host and the transgenic plant got protected for the parasite.

Genetically engineered insulin :

Insulin contains 'A' 'B' and overstrech 'C' polypeptide chain

↓

'C' Polypeptide is removed in mature insulin

↓

Eli Lily prepared two chains corresponding to chains 'A' and 'B' of human insulin

↓

Chain 'A' and 'B' were joined to plasmid to different *E. coli* for replication

↓

Chain 'A' and 'B' were then extracted separately

↓

Separated 'A' and 'B' chains were joined to each other by disulphide bonds.

↓

Mature insulin was obtained.

Gene Therapy : It is a technique of inserting genes into the cells and tissue of an individual to treat a hereditary disease.

- The first clinical gene therapy was given in 1990 to a four year old girl with adenosine deaminase (ADA) deficiency. ADA enzyme is required for proper functioningof immune system.
- This disorder is caused due to the deletion of the gene for adenosine deaminase enzyme. In some children ADA deficiency can be cured by bone marrow transplantation. Lymphocytes from the blood of patient are grown in a culture. A functional ADA cDNA is then introduced into these lymphocytes using retroviral vector. The lymphocytes are transferred into the body of patients.

- As these cells are not immortal, the patient required periodic infusion of such genetically engineered lymphocytes.
- If a functional gene is Introduced into a bone marrow cells at early embryonic stage. It could be a permanent cure of ADA deficiency.

Vaccine Production

Vaccine are used to protect many infectious diseases such as small pox, cholera, Hepatitis B. These are made up of killed or weakened pathogens like viruses and bacteria.

Vaccines are commonly produced through cell cultures or animals or recombinant DNA technology.

Vaccine production involves the following steps.

(i) Generating the antigens : The antigens are generated from the microbes. Virus are grown in primary cells *i.e.*, chicken egg (influenza vaccine) or on continuous cell lines *i.e.*, Human Cultured cells (Hepatitis B). Bacteria against which the vaccines are developed may be grown in bioreactors (Hib Vaccine)

(ii) Isolation of antigens : Antigen are isolated from the cells used to generate it.

(iii) Vaccine is made by adding adjuvant (to increase immune response of antigen), Stabilizers (to increase storage life) and preservativesto allow for the use of multi-dose vials).

Production of Vaccines through Recombinant DNA Technology : Injectable and edible vaccines may be produced through recombinant DNA technology.

- Gene for antigen is isolated from pathogen like Virus.
- This desired gene is introduced in the host cells (yeast).
- Antigen gene is incorporated with genetic material of host.
- The host cell is allowed to grow in the culture.

Agrobacterium tumefaciens is commonly used to deliver the antigen genes into plant cells. Antigens are produced in the plant cells. The edible part of the plants can be consumed to get vaccinated. The transgenic crop plants have the capability to produce vaccine at larger scale and cheaper price.

Stem Cells Technology

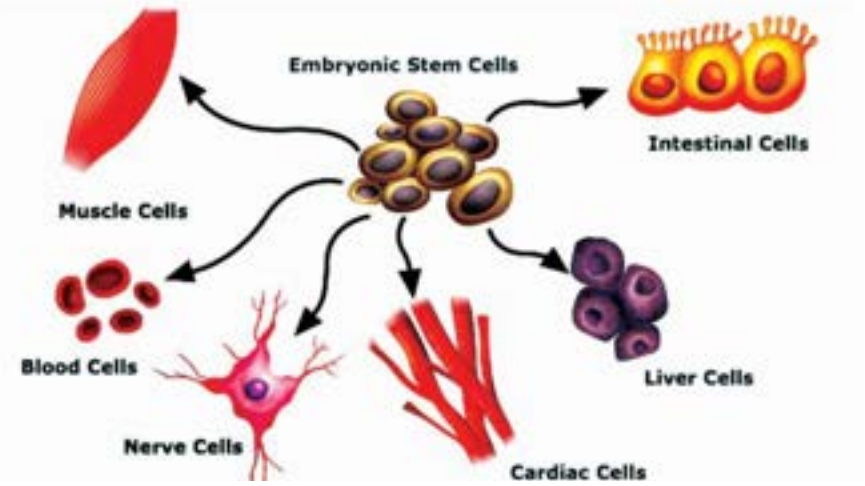
Stem Cells are undifferentiated cells which are able to grow into any type of tissue with specialized function. Stem cells are involved in development, growth and repair in multicellular organisms. Stem cells are used to treat many diseases such as type-I diabetes, heart diseases, cancer, Spinal injuries, arthritis, muscular dystrophy, Alzheimer. It can also be used to make new organs like heart, liver, kidneys, skin, even to produce transgenic animals.

Sources of Stem Cells : Stem cells can be obtained from inner cell mass of embryos, from bone marrow, umbilical cord and amniotic fluid.

There are three Categories of stem cells :

1. **Embryonic stem cells :** The embryo cells are removed easily and cultured in laboratory.
2. **Tissue stem cells :** Bone marrow stem cells can be used to produce bone or cardiac muscle cells.
3. **Reprogrammed stem cells :** Adult special cells are reprogrammed to act as embryonic cells with the help of genetic engineering. Organs for transplantation are developed by this technique.

- Embryonic stem cells have the ability to differentiate into any at the three germ layers-ectoderm, mesoderm or endoderm.
- These cells are isolated from inner cells mass of the blastocyst, 4 to 5 days after *in vitro* fertilisation of an egg.
- The cells are cultured and allowed to grow into cell lines.



The transgenic animals can be produced by stem cell technology the stem cells are isolated from the embryo of selected animal and the desired gene is inserted into these cells. Then, these cells are incorporated in the embryo of host. The embryo is now implanted into the uterus of host animal to grow normally.

Transgenic animals : The animals which carry foreign genes are called transgenic animals.

Steps to produce transgenic animals :

1. Identification and isolation of desired gene.
2. Selection of proper vector or direct transmission of desired gene.
3. Combining of desired gene with the vector using ligase enzyme.
4. Introduction of vector in cells/tissue/embryo/mature individual.
5. Expression of foreign gene in transgenic animal.

Advantages of transgenic animals :

1. Transgenic animals are used to produce the biological products. For example, Rosie (First transgenic cow) produced human alpha-lactalbumin protein enriched milk which was more balanced product for human babies than natural cow-milk.
2. Transgenic mice are used in testing of the vaccine safety before these vaccines are used on humans e.g. Polio Vaccine.
3. Transgenic animal are used to test the toxicity of substances.
4. These animals are used to study how genes contribute to the development of disease and also treatment. Example : cancer, Alzheimer's etc.
5. These animals are used to study the regulation of genes and their affect for normal functioning of the body and its development.

Patent : Patent is a set of exclusive right granted by a state (National Government) to an inventor or their assignee for a limited period of time to prevent others from commercial use of his invention. Biopatents are granted for biological entities and for products derived from them.

Criteria for grant of patents:-

1. **Novelty :** It implies that the innovation must be new.
2. **Non-obviousness :** It implies that it may not be documented but is otherwise well-known.

3. **Utility :** The discovered fact or product should be of a particular use for humans.

Controversies in India regarding patent and biopiracy :

Turmeric : In 1995, the US patent office granted a patent to the University of mississippi medical centre for "Use of Turmeric in wound healing". Dr. R.A. Mashelkar, an Indian scientist challenged the patent. It was established that the use of turmeric as healing agent was well-known in India for centuries and the patent was revoked.

Neem : The European Patent office, Munich granted a patent to the firm of W.R. grace & Co. for 'Fungicidal uses of neem oil'. The patent had been granted on an extraction of oil technique but not on the neem tree itself. In 1996, Vandana Shiva and Ajay Phadke who had rereared neem in India, challenged the patent. Legal action was followed by India Government. Finally, the patent was revoked in 2005.

Basmati Rice : Basmati Rice is a variety of rice which is distinct for its unique aroma and flavour. In India, 27 varieties of basmati are grown.

In September 1997, a Texas company patented Basmati rice lines and grains through the US patent and trademark office. This act caused diplomatic crisis between India and US. Later due to revised decision by the United State patent office, theTexas company lost most of the claims of the patent. This was a case of biopiracy.

Questions

VSA **(I Mark)**

1. Which recombinant vaccine is currently being used in vaccination programme?
2. Name the technique based on the principles of antigen-antibody interaction used in detection of a virus (HIV).
3. The first transgenic cow, produced human protein - enriched milk. Name the cow and the protein found in milk.
4. The insulin produced using recombinant DNA technology is more advantageous than the insulin extracted from pancreas of slaughtered cattle and pigs. How ?

BIOTECHNOLOGY AND ITS APPLIANCES
MULTIPLE CHOICE TYPE QUESTIONS

5. Bt toxin is a

a) Intracellular lipid

b) Intracellular crystalline protein

c) Extracellular crystalline protein

d) Lipid

6. Cry-I endotoxins obtained from Bacillus thuringinusis are effective against.

A) Nematodes

B) Mosquitoes

C) Flies

D) Bell worn

7. In Bt Cotton, the Bt toxin present in plant tissue as protoxin in converted into active toxin due to.

A) Acidic Ph of the insect gut

B) Alkaline Ph of the insect

C) Presence of conversion factors in insect gut

D) Action of gut micro organism

8. The process of RNA interference has been used in the development of plants resistant to.

A) Insects

B) Nematodes

C) Fungi

D) Viruses

9. C-peptidle of Human insulin is :

A) A part of mature insulin molecule

B) Responsible for formation of disulphide bridges

C) Removed during maturation of proinsulin to insulin

D) Responsible for its biological activity

10. Assertion : Bacillus thuringiensis forms protein crystals during particular phase of growth.

Reason : There crystal contain a toxic insecticidal protein that kills the certain insects.

a) If both assertion and reason are true and reason is the correct explanation of assertion.

b) If both assertion and reason are true but reason is not the correct explanation of assertion.

c) If assertion is true but reason is false.

d) If both assertion and reason are false.

11. Assertion : For toxicity testing, transgenic animal are made to carry genes which make them more sensitive.

Reason : Transgenic animals are exposed to toxic substance to be study to know their effect.

A,B,C,D are some as in above question

12. Gene therapy is most recent and advanced therapeutic treatment of defective gene. That has been diagnosed in a child/embryo. It involves insertion of normal healthy and functional gene into a person cells and tissues to treat the

Q12.

i) Gene therapy is "

a) Correction of faulty gene

b) Recovery of faulty gene

c) Addition of a new gene

d) Mutation of a gene

ii) Temporary treatment is to culture :

a) Erythrocyte

b) Lymphocytes

c) Thrombocyte

d) All of the above

iii) Permanent cure of ADA is :

a) Culturing of the cells

b) Transfer of new gene

c) Bone marrow transplantation at embryo stage

d) Culturing of enzyme

iv) ADA deficiency is a :

a) Sexual disorder

b) Mental disorder

c) Physical disorder

d) SCID

SA-I **(2 Marks)**

13. Can a disease be detected before the appearance of its symptoms ?
14. How does a probe help molecular diagnosis.
15. GEAC is one of the organization set up by Indian Government. Write its full form. Give its two objectives.

SA-II **(3 Marks)**

16. Some multinational companies and other organisations are using bioresources for commercial benefits, without proper authentication and compensation to concerned authorities.
    (a) Give the term for this unauthorised act.
    (b) Suggest any two ways to get rid of this.
17. A bacterium *Bacillus thuringiensis* produces a toxic protein named 'Cry protein' that is lethal to certain insects but not to bacterium.
    (a) Why this toxin does not kill the bacteria ?
    (b) What type of changes occur in the gut of insects on consuming this protein ?
    (c) How man has exploited this protein for his benefit ?

18. Given below is an incomplete flow chart showing the process of production of nematode resistant tobacco plants based on RNAi technique.

(i) Write the missing steps in proper sequence.

(ii) At which level RNAi silences the gene ?

(a) Splicing of a specific mRNA

↓

(b) .....

↓

(c) Formation of sense and antisense RNA in host cell

↓

(d) .....

↓

(e) Initiate RNA interference

↓

(f) .....

↓

(g) .....

↓

(h) Transgenic plant got protected from parasite.

LA **(5 Makrs)**

19. The clinical gene therapy is given to a 4 years old patient for an enzyme which is crucial for the immune system to function.

(A) Lymphocytes of the Patient.

↓

(B) .....

↓

(C) Introduction of functional ADA cDNA into lymphocytes.

↓

(D) .....

Observe the therapeutical flow chart and give the answer of the following:

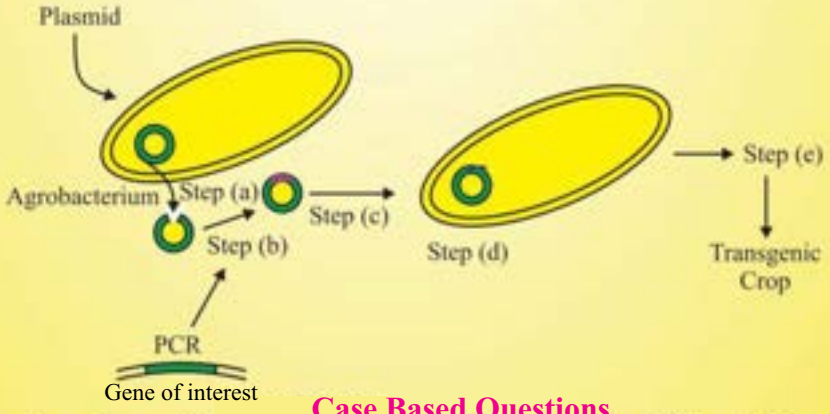
(a) Complete the missing steps (B) and (D)

(b) Identify the disease to be cured.

(c) Why the above method is not a complete solution to the problem ?

(d) Scientists have developed a method to cure this disease permanently. How ?

20. In the given figure, *Agrobacterium* is utilized for the production of a transgenic crop. Explain the steps a, b, c, d and e shown in the figure.



Case Based Questions

21. In the given figure, Form (A) and Form (B) represents different forms of a proteinaceous hormone secreted by pancreas in mammals.

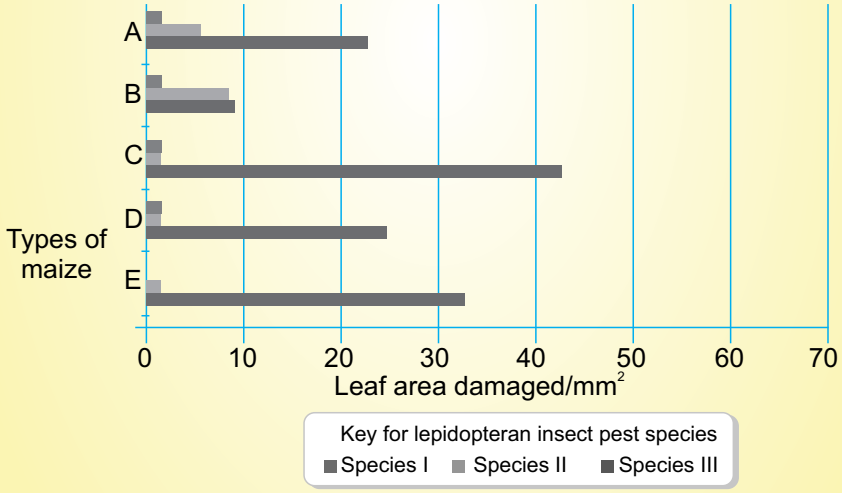
Form (A) Form (B)

(a) Name the hormone. What type of bonding is present between chains of this hormone ?

(b) What are these form (A) and form (B)? How these forms differ from each other ?

(c) Explain how was this hormone produced by Eli Lilly, an American company, using rDNA technology.

22. Insects in the Lepidopteran group lay eggs on maize crops. The tarvae on hatching feed on maize leaf and tender cob. In order to arrest the spread to three such Lepidopteran pests, Bt maize crops were introduced in an experimental field.



A study was carried out to see which of the three species of lepidopteran pests was most susceptible to Bt genes and its product.

The lepidopteran pests were allowed to food on the same Bt-maize crops grown on 5 fields (A-E).

The graph below shows the leaf area damaged by these three pests after feeding on maize leaves for five days.

Insect gut pH was recorded as 10, 8 and 6 respectively for Species I, II and III respectively.

(a) Which of the three species is least susceptible to Bt toxin?

(b) Name the gene used to make Bt-maize and its source organism.

(c) Why is the effect of Bt toxin on lepidopteran species not similar in the three species?

(d) How does the Bt toxin kill the insect, but not the bacterium?

Answers

VSA **(1 Mark)**

1. Hepatitis B recombinant vaccine.
2. ELISA (Enzyme linked immuno-sorbent Assay)
3. Rosie, alpha-lactalbumin
4. Insulin obtained from animal source causes allergy.

SOLUTION LESSON 12

MCQ

5. B) 6. D) 7. B) 8. B) 9. C) 10. B) 11. d)

Q12 :

i) a ii) b iii) c iv) d

SA-I **(2 Marks)**

13. Yes, early detection of disease is possible by the use of recombinant DNA technology, PCR, ELISA.

14. A single stranded DNA/RNA tagged with a radioactive molecular probe is allowed to hybridise to its complementary DNA in a clone of cells. It followed by detection using autography. The clone having the mutated gene will not appear on the photographic film.

15. GEAC—Genetic Engineering Approval Committee. Objectives of GEAC are :

(i) To make decisions regarding validity of GM research.

(ii) Safety of introducing GMO for public use.

SA-II **(3 Marks)**

16.(a) Biopiracy

(b) (i) Benefits of bio resources should be shared between developed and developing nations.

(ii) Laws should be developed to prevent unauthorised exploitation of the bioresources.

17.(a) In bacteria, cry protein remains in inactive form as Prototoxin.

(b) Prototoxin becomes active toxin in alkaline pH of gut of insects. Toxins bind to surface of midgut and cause perforation, swelling, lysis of cells ultimately leading to death of insect.

(c) Specific Bt toxin genes isolated from *Bacillus thuringiensis* and incorporated into several crop plants such as cotton and corn which become pest resistant against certain insects.

18.(i) (b) Using *Agrobacterium* as a vector, introduced into tobacco

(d) dsRNA (double stranded RNA)

(f) Silenced specific mRNA of the nematode

(g) Parasite could not survive.

(ii) RNAi silences the gene at translation level

LA **(5 Marks)**

19.*Step* (B) : Lymphocytes are grown in culture medium.

Step (D) : Infusion of genetically engineered lymphocytes into patients.

(b) Adenosine deaminase (ADA) deficiency.

(c) As genetically engineered lymphocytes are not immortal, the patient requires periodic infusion of cells.

(d) If the gene isolated from bone marrow cells producing ADA is introduced into cells at early embryonic stages, it could be a permanent cure.

20. Step (a) Plasmid is removed and cut open with restriction endonuclease.

Step (b) Gene of interest is isolated from another organism and amplified using PCR.

Step (c) New gene is inserted into plasmid

Step (d) Plasmid is put back into *Agrobacterium*

Step (e) *Agrobacterium* based transformation.

21. (a) Insulin, Disulphide bonds

(b) Form (A) : Proinsulin

Form (B) : Mature insulin.

Proinsulin contains an extra stretch called C - peptide which is absent in mature insulin.

(c) Eli-Lilly company prepared two DNA sequences corresponding to A and B peptide chains of human insulin and introduced them in plasmid *E. coli* to produce insulin chains. Chains A and B were produced separately, extracted and combined by creating disulphide bonds to form insulin.

22. (a) Species III

(b) — Cry gene

— Source: Bacillus thuringiensis.

(c) pH in the got of insects is different and toxin gets activated at optimum pH.

(d) Bt toxin gets activated in gut of insects and absent in bacterium.