29

BIOTECHNOLOGY

At home we prepare food items such as yoghurt (curd), cake, bread, idli and dosa etc. by the action of tiny microorganisms, the bacteria and fungi. Brewers use yeast (a kind of fungus) to make beer. Antibiotics such as penicillin are obtained from certain fungi. Nowadays, biological processes such as fermentation by microorganisms is being used in industry on a commercial scale for making food, drinks, drugs (medicines) and industrial chemicals. Modern techniques in biotechnology are programming microorganisms for this task. In this lesson, you will learn about use of microorganisms in industries.

OBJECTIVES

After completing this lesson, you will be able to:

- *appreciate the importance of biotechnology in human welfare;*
- *explain the use of biotechnology in industry;*
- *list the microbes used in the industry and the products manufactured through their use;*
- *explain fermentation and outline the process of making alcohol by using microorganisms;*
- *describe the process of making yoghurt and cheese on a large scale;*
- *explain the contribution of microorganisms in making antibiotics and vaccines;*
- *define genetic engineering and mention its utility;*
- *define transgenic organisms, mention the steps in their production and cite a few examples of transgenic plants and animals;*
- *explain the process and importance of gene therapy;*
- *explain bioremediation and biopesticides.*

29.1 BIOTECHNOLOGY

The word biotechnology has come from two words namely **bio** (meaning biology) and **technology** (meaning technological application). Thus **biotechnology is defined as the industrial application of living organisms and their biological processes such as biochemistry, microbiology, genetic engineering, etc. in order to make best use of the microorganisms for the benefit of mankind.**

Biotechnology is applied in many areas to produce foods and medicines, in the development of new diagnostic tools, gene therapy, DNA finger-printing for forensic purposes etc.

29.1.1 Applications of Biotechnology

1. Health and medicine

Fighting infectious diseases : Biotechnology is used extensively in the study of infectious diseases such as SARS (Severe Acute Respiratory Syndrome), influenza, etc. As a result more effective pharmaceuticals have been developed.

Development of vaccines and antibiotics : Using technology, microorganisms are used to develop antibiotics and vaccines to cure diseases. For example, bacteria *Bacillus polymysea* is used to produce polymyxin B antibiotic (used to cure urinary tract infections), fungus *Penicillium notatum* is used to produce penicillin (used to cure fever, pneumonia, etc.)

Treating genetic disorders : Disease can occur when genes become defective due to mutations. With advance in biotechnology it will in the near future be possible to use gene therapy to replace an abnormal or faulty gene with a normal copy of the same gene. It may be used to treat ailments such as heart disease, inherited diseases such as SCID, Thallesemia.

In forensic science : With the help of new techniques such as **DNA fingerprinting**, it has now become easy to identify criminals and have many other applications.

2. Environment

Cleaning up and managing the environment : Cleaning up the environment using living organisms is called **bioremediation**. Naturally occurring, as well as genetically modified microorganisms, such as bacteria and fungi, and enzymes are used to break down toxic and hazardous substances present in the environment.

3. Agriculture

Biotechnology has helped in production of crops with improved disease resistance; herbicide-tolerance and insecticide-resistance. Plants with improved nutritional value for livestock etc. have also been bred through biotechnology.

Control of pests : One application of biotechnology is in the control of insect pests. The genetic make-up of the pest is changed by causing some mutations. These pests become sterile and cannot produce next generation.

Manufacturing and bio-processing : With the help of new biological techniques it has become possible to grow on large scale, the plants that produce compounds for use in detergents, paints, lubricants and plastics etc.

Food and drinks : With biotechnology, it has now become easy to process foods and their products. Preservation and storing of food for consumption later has become easy and cheap with the help of biotechnology. Seedless grapes and seedless citrus fruits have been developed using biotechnology.

4. Industry

Biotechnology has been used in the industry to produce new products for human consumption. Food additives have been developed which help in the preservation of food. Microorganisms are used in the mass production of items such as cheese, yoghurt, alcohol, etc.

29.1.2 Industrial Microorganisms and Their Industrial Products

Important microorganisms used in industries include

- yeasts (fungi)
- moulds (fungi)
- bacteria
- filamentous bacteria (*actinomycetes*)

Microbes are used in the manufacture of several products. Some of these are

- alcohol-containing beverages
- yoghurt (curd)
- proteins
- antibiotics and monoclonal antibodies
- vitamins, steroids and enzymes
- biogas

The progress in gene manipulation and genetic engineering has introduced the use of cultured mammalian cells and 'hybridomas' in the industries. Hybridomas are created by fusion of cells belonging to organisms of different species.

29.1.3 Production of Alcohol – Containing Beverages

Fermentation

Fermentation is a process by which carbohydrates such as sugar are converted into alcohol.

$$\text{Glucose} \xrightarrow[\text{enzyme}]{\text{yeast}} \text{Ethyl alcohol} + \text{Carbon-dioxide} + \text{ATP}$$

Yeast and bacteria are capable of fermenting sugar to alcohol. Fermentation is an energy yielding process.

In the mid nineteenth century, Louis Pasteur showed that fermentation by the yeast *Saccharomyces cerevisiae* yields beer and buttermilk. Presently yeast is being used on a large scale for fermentation.

Bakers use yeast to leaven (raise) dough to make bread. Yeast is grown on molasses and is also packed and sold. Yeast is used to raise cakes and bread while baking.

Alcoholic beverages are manufactured by fermentation of sugars by the yeast, *Saccharomyces cerevisiae*. It is called **Brewer's yeast**. The source of carbohydrate fermented by yeast gives the beverage its specific flavour. For example :

– Wine is obtained by fermentation of grapes. Grapes are fermented by *S. cerevisiae* and its soluble sugars (glucose and fructose) are converted into CO_2 and ethyl alcohol.

– Fermentation is carried out in large tanks called bioreactors.

– Barley malt is fermented to yield beer.

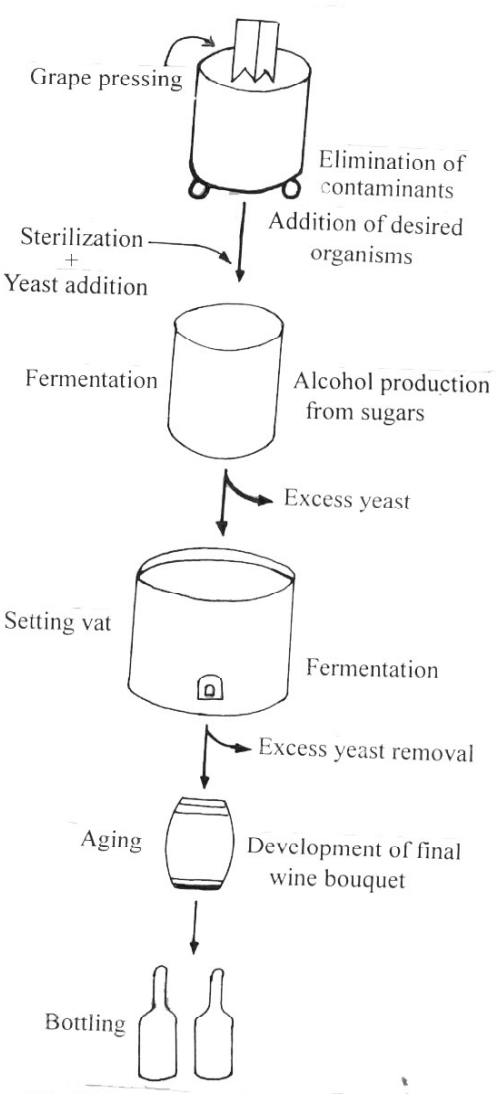


Fig 29.1 Obtaining wine from *grapes by fermentation by* Yeast

Steps taken for fermentation

(i) Fermenter or tank and the nutrient medium are sterilised by steam under pressure (autoclave).

(ii) The correct strain of yeast is selected.

(iii) The yeast is inoculated into the medium. Inoculation can be done in two ways:

(a) Yeast can be grown as a layer on the surface of nutrient medium. This is called **support growth system**.

(b) Cells or mycelia are suspended in a liquid medium. This is called **suspended growth system**.

(iv) Care is taken to maintain the right temperature, pH, oxygen and carbon-dioxide concentration.

(v) The medium is stirred and left to ferment.

(vi) The sugar in the medium gets fermented by enzymes released by yeast.

(vii) The fermented product is taken out (Fig. 29.1).

Some alcohols manufactured by yeast fermentation are : Ethyl alcohol, butanol and glycerol. The same method also yields **lactic acid** and **acetic acid** (vinegar).

The yeast extract left after removal of the beverage can be used as animal feed. It is also a rich source of vitamins.

INTEXT QUESTIONS 29.1

1. Name three different kinds of microorganisms used in the manufacture of industrial products.

   .....

2. Name three products obtained in industries by using microorganisms.

   .....

3. Name two alcohols produced through fermentation by yeast.

   .....

4. Name the two methods of inoculation of yeast in the medium.

   .....

5. Match the items given in columns A with those given in column B.

Column A	Column B
1. Bioreactor	(a) Butanol
2. Steaming under pressure	(b) Fermentation tank
3. Alcohol	(c) Autoclave

29.2 YOGHURT AND CHEESE MAKING

At home we add a bit of yoghurt (starter) to milk and it sets. The milk becomes yoghurt or curd due to the milk curdling enzymes released by the increasing population of bacteria, *Lactobacillus* present in the starter. (Table 29.1) On a commercial scale, making of yoghurt as well as cheese utilises **Rennet** tablets for this purpose. Rennin is the milk curdling enzyme obtained from the calf stomach. This method is not so popular any more.

Whether by bacteria or by “rennin” when milk is ‘curdled’, milk protein casein, separates from the liquid which is called whey. Lactic acid bacteria convert lactose in the milk into lactic acid which lowers the pH. Lowered pH causes souring which is essential for preservation.

Butter can be made by violently shaking (churning) sour milk. The fat globules separate and form butter. A starter culture of *Streptococcus cremosis, Leuconostoc is* added to the milk when butter, yoghurt or cheese are made.

Table 29.1 Fermenting microbes used for dairy products

Fermented product	Fermenting microorganism	Description
Yoghurt	*Streptococcus thermophilus* and *Lactobacillus bulgarians*	Product made from low or non-fat milk and stabilisers like gelatin added.
Butter	*Lactococcus lactis*	Cream is incubated till desired acidity achieved followed by churning, washing and salting

29.2.1 Microorganisms and antibiotics

In 1928, Alexander Fleming accidentally discovered that one microorganism can inhibit the growth of another organism. Selman Waksman 1942 coined the term antibiotic (anti: opposed to, biotic: living organism)

> Antibiotic is a substance produced by a microorganism such as bacteria or fungi which inhibits the growth of another microorganism. Antibiotics are generally small molecules with a molecular weight less than 2000 Da. They are not enzymes. The antibiotic interferes with the vital metabolic processes of the pathogenic bacterium and prevents its growth and reproduction.

Wide-spectrum and narrow-spectrum antibiotics

Modern medicines have found a specific antibiotic for almost every different pathogen. *Streptomyces* bacterium yields some of the most widely used antibiotics like Chloramphenicol, Erythromycin, Tetracycline etc. These are called '**broad spectrum antibiotics**' and can be used against more than one kind of pathogenic bacterium. Streptomycin and Penicillin are **narrow spectrum antibiotics** used against few pathogenic bacteria.

Drawbacks of antibiotics

Use of antibiotics was a big step in curing infectious diseases - a safe, sure and relatively inexpensive cure. But even now we find many people suffering from bacterial diseases. The reasons for this are:

1. Some people are allergic to a particular antibiotic.
2. Some disease causing bacteria undergo mutation and become resistant to a particular antibiotic to which they were earlier sensitive.

Sources of antibiotics

Some of the common antibiotics and their source organisms are given in table 29.2.

Table 29.2 Major antibiotics and their sources

Antibiotic group	Source
Tetracyclin	*Streptomyces sp*
Chlorotetracycline	*Streptomyces auriefaciens*
Chloramphenicol	*S. venezuelae*
Cycloheximide	*S. griseus*
Streptomycine	*S. griseus*
Cephalosporin	*Cephalosporium acremonium*
Penicillin	*Penicillium chrysogenum*

29.3 VACCINATION

In 1790, Edward Jenner observed that milkmaids did not get smallpox as they were exposed to a milder disease cowpox. Jenner infected a boy with cowpox germs and after two months with small pox germs. The boy did not get small pox. Jenner proposed that if mild or attenuated (weakened) germs were introduced into the body, they would not cause the disease. He gave the term **vaccine** (latin *vacca* : cow) or vaccination, for the weakened germ and its protective inoculation.

Today, the principle of vaccination has been extended to prevent attack of many diseases. When vaccines are made from attenuated disease causing bacteria, they are termed “first generation vaccines”. The “second generation vaccines” have been produced by genetic engineering or recombinant DNA technology about which you shall study in the next section. Second generation vaccines for Hepatitis B virus and *Herpes* virus are already in use. Vaccines synthesised from chemicals are termed “third generation vaccines”.

29.4 PRODUCTION OF VITAMINS

Vitamins are nutrients required in very small amounts for essential metabolic reactions in the body. Vitamins are produced using biotechnology. Vitamin C was the first vitamin to be produced during a fermentation process by using bacteria. B_{12} or cyanocobalamin and B_2 or Riboflavin were obtained from liver extract. The production of B_{12} involved fermentation by propionic bacteria. In nature B_2 is found in cereals, vegetables and yeast but the yield of B_2 can be enhanced hundred to three hundred fold by using microbes.

29.5 PRODUCTION OF BIOGAS

Biogas is a new conventional source of fuel. Its use can save fossil fuel (coal, kerosene, petrol etc.) which are fast getting depleted.

Biogas is made from organic waste including faecal matter. Cowdung or faeces have lignocellulose. The energy used as fuel comes from methane (CH_4). Cowdung forms the primary source of biogas. In India cowdung is available in plenty in villages and small scale methane generating plants have been designed.

Any biodegradable (that which can be decomposed by bacteria) substance can be fermented anaerobically (in the absence of oxygen) by methane-producing (methanogenic) bacteria. Cowdung or faeces are collected and put in a biogas digester or fermenter (a large vessel in which fermentation can take place). A series of chemical reactions occur in the presence of methanogenic bacteria (CH_4 generating bacteria) leading to the production of CH_4 and CO_2.

While generating biogas, few fermentation parameters have to be maintained. These are as follows:

1. Fermentation should be in an anaerobic environment and no free Oxygen should be present.
2. pH in the fermenter should be close to neutral, around 6.8 to 7.6
3. Methanogenic bacteria are to be used for fermentation.

Several kinds of reactors have been designed. One side of the reactor is for input, that is, for introducing cowdung or faecal matter into the reactor. Other side of the reactor has an outlet for removal of biogas: What is left behind is called **slurry**. Gas gets stored above the slurry level. Slurry forms excellent manure.

Advantages of biogas

1. Biogas is a fuel used to cook food, light lamps etc.
2. Slurry left after biogas production forms a soil conditioner (manure).
3. Biogas is much cheaper than LPG (Liquefied Petroleum Gas) which we commonly use these days in our houses.

INTEXT QUESTIONS 29.2

1. Name the bacterium responsible for curdling of milk.

   .....

2. Who discovered antibiotics?

   .....

3. What do you mean by second generation vaccines?

   .....

4. Which was the first vitamin to be produced by fermentation?

   .....

5. Which bacteria cause the production of biogas?

   .....

29.6 GENETIC ENGINEERING

An engineer fixes a machine to make it work efficiently. Body is like a machine and genes, the nucleotide sequences in DNA have the information for products to run this machine. With progress in molecular biology, techniques have been developed by which a scientist can now manipulate genetic material, replace genes

or replace gene products in the body, make identical copies of these genes and store them in a gene library. This is called **genetic engineering**.

29.6.1 Importance of genetic engineering

You know that diabetes mellitus is a genetic disorder. A diabetic patient lacks a gene which has the information for synthesis of insulin, so such a person cannot secrete insulin. Take another example. A person suffering from **Thalassemia** lacks the gene for haemoglobin and can survive only through frequent blood transfusions. A person suffering from sickle cell anemia has an altered gene whose product makes the red blood corpuscles abnormal on exposure to oxygen because they contain faulty haemoglobin.

Human suffering from genetic disorders such as those cited above have now hope in genetic engineering. Genetically engineered copies of DNA can be produced and stored in gene libraries to be used when required.

In the previous sections of this lesson you have studied about the use of microbes to produce various products on a commercial scale. Currently bacteria are being genetically manipulated to act as biological factories to produce various kinds of proteins such as enzymes, hormones, antibodies through genetic engineering. Researchers have isolated genes which can be used to produce effective vaccines.

Researchers have also developed bacterial strains, through genetic manipulation, which can degrade harmful environmental pollutants.

29.6.2 Recombinant DNA technology

Genetic engineering may be defined as construction and utilisation of new DNA molecules that have been engineered by recombinant DNA techniques. **The technique of genetic engineering is in the production of recombinant DNA.** Recombinant DNA, as the name suggests, involves cutting a piece of original DNA and inserting in its place a different segment of DNA. The recombined or recomposed DNA is then copied multifold inside bacterial cells and stored in a gene library for use when required. The multiple copies of the gene are termed **cloned DNA or cloned genes.**

Causing genetic change by artificially manipulating DNA is genetic engineering.

Clone is a group of genetically identical cells. Such cells are descendents of a single cell. When a bacterium with recombinant DNA divides several times, it provides a clone containing a specific segment of DNA from another species.

The production of genetically identical individuals or genetic material from a single cell is called cloning.

Recombinant DNA technology resulted from the two discoveries made while experimenting with bacteria :

(i) presence of plasmids or extra chromosomal DNA fragments in the bacterial cell which replicate along with bacterial DNA and can be used as a vector for carrying foreign DNA.

(ii) presence of specific restriction enzymes which attack and cut DNA at specific sites.

29.6.3 Tools and steps in recombinant DNA technology

Recombinant DNA technology is a "cut and paste" technology. Specific nucleotide sequences are cut from the DNA of humans, other animals or plants and "pasted" into plasmids. DNA of the plasmid carrying nucleotide sequence of another organism is the recombinant DNA. It is then inserted into bacteria. Bacteria divide repeatedly and a clone of bacteria with the recombinant DNA is obtained.

Five requirements for recombinant DNA technology are:

(i) Cell culture
(ii) Restriction endonuclease enzyme
(iii) Plasmids
(iv) Ligases
(v) Host bacteria

(i) Cell culture : Cultured cells of an animal or plant (or even a bacterium) carrying the required gene (nucleotide sequence of DNA) in its nucleus.

(ii) The enzyme Restriction endonuclease : Restriction endonucleases cut short specific DNA sequences. There are many different restriction endonucleases found in bacteria. Each of these enzymes very specifically recognises a particular DNA sequence (usually 4 to 6 bases) and cuts it. These enzymes are the "molecular scissors". Either they cut both the strands at the same place or at different places so that the two DNA strands hang out at the two ends. Two cuts at the two ends of a DNA segment releases the cut part as the restriction fragment. The ends are single stranded and called sticky ends.

Thus a piece of DNA containing a particular gene can be obtained by selecting a particular restriction endonuclease.

(iii) Plasmids : Plasmids are extra chromosomal DNA molecules in a bacterial cell which have sequences matching those of the required gene and can be similarly cut by the same restriction enzymes. Plasmids can readily enter bacteria, yeast or other speedily reproducing cells.

(iv) DNA ligase : It is an enzyme which can seal one DNA fragment with another DNA segment, both having sticky ends. Ligase is the "molecular glue".

(v) Host Bacteria : Host bacteria are the bacteria whose plasmid is used for carrying foreign DNA.

Sequences of steps in recombinant DNA technology:

1. Specific restriction enzyme is selected.
2. Cell culture with required gene in the cells is obtained.
3. Restriction enzyme cuts the DNA at two ends of the specific gene and a restriction fragment is obtained (Fig. 29.2 a, c)
4. Same restriction enzyme cuts a matching DNA sequence from a plasmid (Fig. 29.2 b, d)

5. Ligase joins the restriction fragment in the place vacated by the cut DNA segment of the plasmid. The plasmid becomes a recombinant plasmid containing a foreign DNA fragment (Fig. 29.2 e, f). Its DNA is the recombinant DNA. Since plasmids can carry foreign DNA, they are called **clonal vectors**. Bacteriophages (viruses) can also function as clonal vectors.
6. The recombinant plasmids then enter the bacteria.
7. Bacteria divide. Recombinant plasmids replicate along with bacterial DNA.
8. A large population of bacteria (more than a million) containing recombinant DNA can be obtained in less than ten hours.
9. Multiple identical copies of DNA fragments inserted into plasmids or bacteriophage (bacterial virus) then obtained and preserved in a DNA library.
10. These DNA fragments are the cloned DNA.

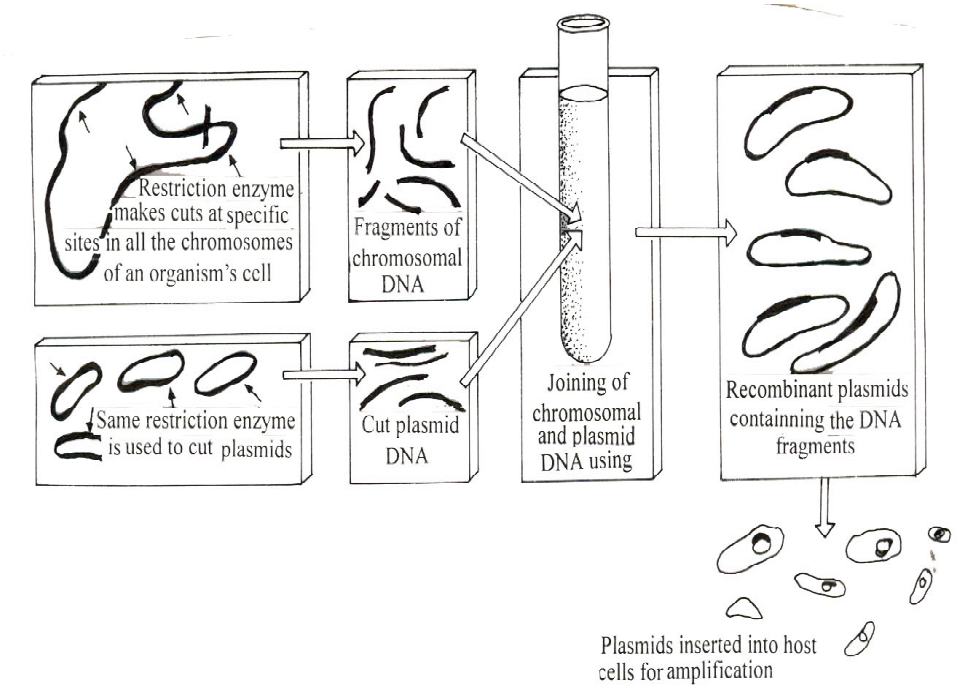


Fig. 29.2 Steps in formation of multiple copies of recombinant DNA for DNA library

INTEXT QUESTIONS 29.3

1. Define genetic engineering.

   .....

2. What is a clone?

   .....

3. What do you mean by the term recombinant DNA?

   .....

4. Where are plasmids found?

   .....

5. Why are restriction enzymes called "molecular scissors"?

   .....

6. Name the enzyme which joins DNA fragments.

   .....

7. What is a clonal vector?

   .....

8. What do you mean by a recombinant plasmid?

   .....

29.6.4 Applications of genetic engineering

1. Protein manufacture

You would recall from earlier section of this lesson that bacteria and yeasts have been used for centuries to produce cheese, alcohol, etc. and more recently antibiotics, etc. Currently, plasmids in bioengineered bacteria carry some human genes and these genes are expressed to give large quantities of human proteins which are clinically useful. The development *of* recombinant DNA technology and gene cloning has generated a new industry for manufacturing proteins. Earlier valuable proteins could be obtained from eukaryotes in small amounts and at heavy expense, but now these can be produced in large quantities. For example, until sometime back growth hormone was available only in tiny amounts and was extremely expensive as it had to be extracted from endocrine glands. Today, it can be made available in large quantities through recombinant DNA technology. In 1982 production of human insulin became the first commercial success of recombinant DNA technology.

There are several proteins of therapeutic (medical) value which are available now through recombinant DNA technology. These are cloned human gene products approved for use or being developed. Following table 29.3 gives the names and uses of some of these:

Table 29.3 The names of proteins and their uses

Protein	Used in
1. Insulin	Diabetes mellitus
2. Growth hormone	Pituitary dwarfism
3. Erythropoietin	Anaemia
4. Interferons	viral infections
5. Interleukin 2	Cancer
6. Clotting factor VIII	Haemophilia A
7. Clotting factor IX	Haemophilia B
8. Monoclonal antibodies	Infectious diseases
9. Tissue Plasminogen factor	Heart attack

2. **Enzymes** have also been produced from cloned genes. The following table 29.4 gives the names of such enzymes and their uses:

Table 29.4 The names of enzymes and their uses

Enzymes	Used in
Proteases	manufacture of detergents, meat tenderisers.
Amylases	manufacture of beer, bread and textiles
Glucoisomerases	to make corn syrup, which is sweeter than sucrose and used to flavour soft drinks

Enzymes are fragile and have to be entrapped in gel and encapsulated in small artificial cells.

3. Antibiotics

Since the discovery of Penicillin in 1920s, more than 6000 antibiotics have been isolated from various microorganisms and have resulted in an enormous improvement in human health. Research is in progress to genetically engineer biosynthetic pathways for the synthesis of antibiotics. Novel antibiotics have also been obtained through genetic manipulation.

4. Vaccines

Bioengineered vaccines have been developed for rabies and hepatitis B. A gene for the antigen protein is inserted into a plasmid and the bacteria containing recombinant DNA then generate large quantities of the protein. The protein is added to the vaccine. Antibodies immediately form against the antigen when vaccinated.

INTEXT QUESTIONS 29.4

1. Name any two proteins and two enzymes obtained by recombinant DNA technology.

   (i) ..... (ii) .....

2. How is recombinant DNA technology useful for pharmaceutical companies?

   .....

3. Name any two diseases for which bioengineered vaccines have already been developed.

   (i) ..... (ii) .....

29.8 TRANSGENE AND TRANSGENIC

Genetic engineering has made possible production of organisms of one species carrying genes of another species. The foreign gene is called a **transgene.** The plant or animal carrying it is termed as **transgenic.**

Genetically engineered organisms carrying foreign genes are termed transgenics.

1. Usefulness of transgenic organisms

1. For a better yield desirable traits can be introduced or increased in agricultural plants and domestic animals, especially the cattle.
2. Valuable products can be produced by transgenic plants and animals.
3. Transgenic plants and animals can be used for investigating biological processes such as gene expression.

2. Methodology for production of transgenics

There are two methods which are mostly used for generating transgenics:

(i) Microinjection of foreign DNA into pronuclei of fertilised eggs.

(ii) Retroviral vector method. Infection of pre-implantation embryos with retroviruses carrying foreign DNA.

The first method has the following steps:

(i) Collection of oocytes from the animal killed in slaughter house or surgically from female parent cell.

(ii) In-vitro maturation of oocytes.

(iii) In-vitro fertilisation with male semen.

(iv) Eggs (oocytes) to be centrifuged to concentrate yolk which in normal cells prevents male pronuclei from being seen under the dissecting microscope.

(v) Microinjection of "input DNA" into male pronuclei (Fig. 29.3). Usually hundred to thousand copies of the gene of interest are injected.

(vi) In-vitro development of embryos.

(vii) Non-surgical implantation of one embryo into a recepient foster mother.

(viii) Screening of DNA of the offspring of foster mother for presence of transgenes.

(ix) Offspring with the transgenes are the transgenic organisms.

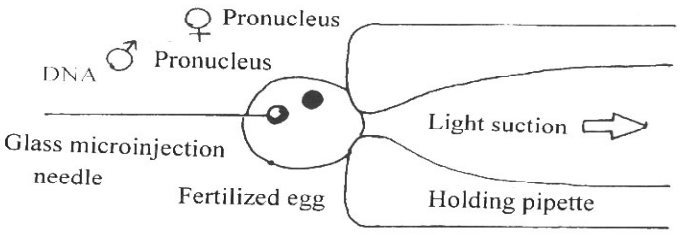


Fig. 29.3 Microinjection of input DNA into male pronucleus

In the **second method** called the **retroviral vector method,** DNA required to be transferred into the female is introduced through the retrovirus which infects the cells of an early stage embryo before implantation into a receptive female.

Transgenic plants

By recombinant DNA techniques, plant breeders can now directly modify the DNA of plants. They can add genes from other species to the plant. The most popular method for doing this is to produce a transgenic plant by the use of *Agrobacterium tumefaciens*. It is a soil bacterium which has a natural "genetic engineering" system. It has a plasmid which can be inserted into plant cells. *Agrobacterium tumefaciens* causes galls (tumours) (Fig. 29.4) in several plants. The information for production of galls is present on a plasmid, (T_1) in the bacterium. A segment of DNA from the plasmid can be transferred into plant cell and transform them. In the T_1 plasmid, gall forming genes can be removed and substituted by deserved genes. The plasmid can then be used to transform plant cells. Such foreign genes in the chromosomes of transformed plant cells can normally be expressed (Fig. 29.4).

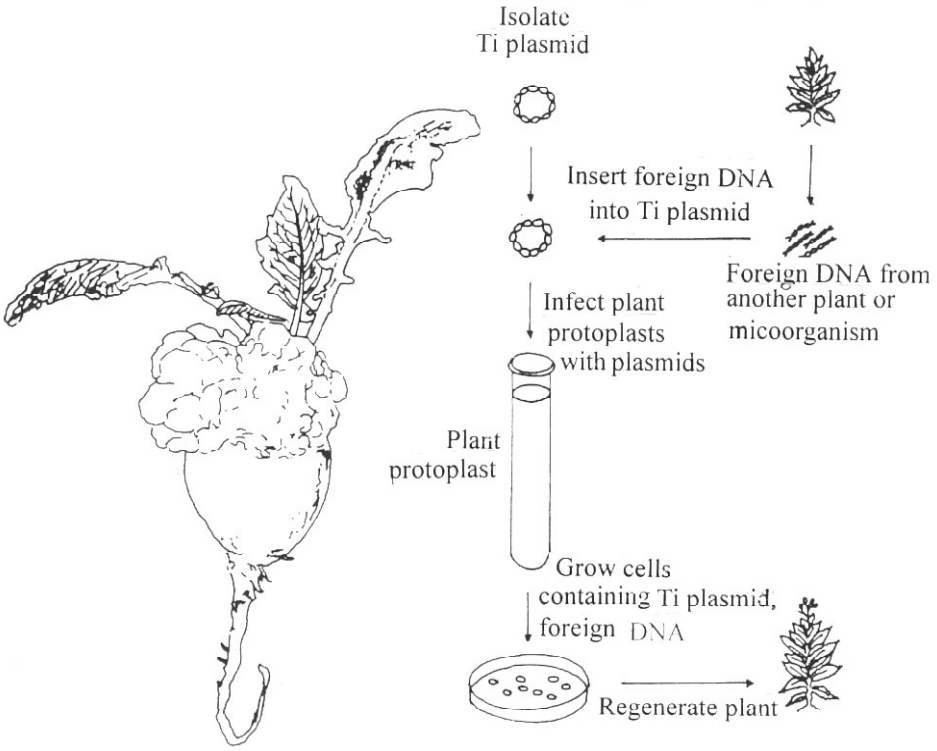


Fig. 29.4 Gall caused on turnips by bacteria carrying T1 plasmid

Examples of transgenic plants

(i) Cotton which can resist attack by worms.

(ii) Corn and soybean which are more tolerant to draught and pesticides.

Transgenic plants can also serve as factories to produce medically and commercially useful proteins. Serum albumin is used in preparations given to patients with burn injuries and others for replacement of body fluid. Genetically altered potato and tobacco plants can yield-serum albumin.

Transgenic animals

Mice : It is difficult to generate transgenic animals as animal cells do not accept plasmids. Transgenic mice are however routinely produced in the laboratories throughout the world by microinjecting foreign DNA. Gene for growth hormone from rats was microinjected into mouse eggs. These mice grew larger than their little mates. This was because rat gene got integrated into mouse DNA and was being expressed. (Fig. 29.5).

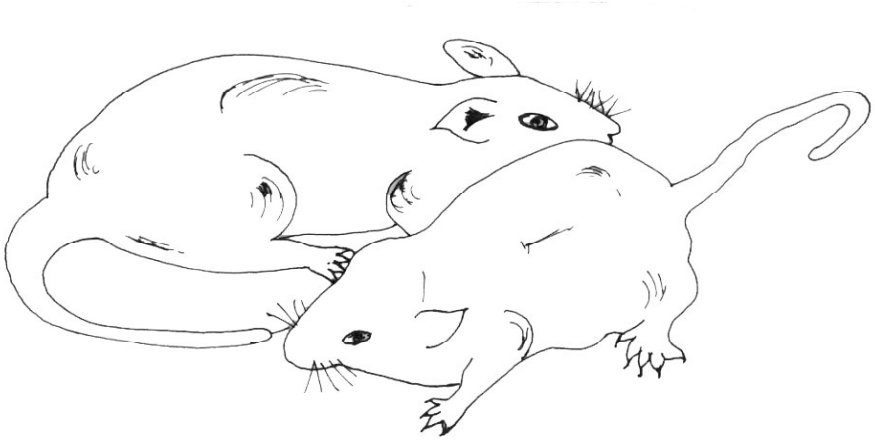


Fig. 29.5 Transgenic mouse compared to normal mouse

Goats : Transgenic goats have been developing from a fertilised egg injected with recombinant DNA consisting of goat gene sequences spliced with human genes for tPA (tissue plasmanogen activator). Goat milk contains this factor which dissolves blood clots. This has proved very useful for heart attack (coronary thrombosis) and stroke patients.

Cattle : Transgenic livestock have the potential to produce large quantities of drugs faster and at much cheaper rates than from bacteria which have to be cultured in huge industrial vessels.

Chinese hamster : Blood clotting factor VIII genes have been inserted in chinese hamster ovary cells. This factor saves the patients suffering from haemophilia A. Blood clotting factor generated through the recombinant DNA technology in Chinese hamster eliminates the need to get it from human blood as also the risk of transmitting AIDS.

29.8.1 Bioremediation (remedy through organisms)

Genetically engineered bacteria can clean up pollutants from the environment. This is called **Bioremediation**. The transformed bacteria metabolically breakdown toxic pollutants to harmless compounds.

Mercury resistant bacteria process metallic mercury (which damages the nervous system) into a nontoxic compound.

INTEXT QUESTIONS 29.5

1. Define a term transgenic.

   .....

2. Name the gall producing bacterium and the plasmid which can be conveniently used to produce transgenics.

   .....

3. What is bioremediation?

   .....

29.9 HUMAN GENE THERAPY

Many people are born with and suffer from diseases such as sickle cell anaemia, haemophilia, severe combined immuno deficiency (SCID), colour blindness etc. Such diseases are caused due to genetic defects. These genetic defects are hereditary. It has been estimated that around 2000 children in India alone are born every day with genetic disorders. Let us learn about the methods of removal and correction of genetic defects.

29.9.1 Gene function

Genes play a number of different roles in the proper functioning of an organism by (a) controlling synthesis of enzymes involved in biochemical reactions, (b) regulating their synthesis such that the right enzyme appears at the right time. Sometimes genes may not function properly due to some irregularity or defect in their structure. This may lead to genetic disorders. A defective gene may appear in an individual in the following two ways:

(i) Certain genes are inherited and the defect runs in the family. For example : Colour blindness, haemophilia, sickle cell anaemia.

(ii) A gene becomes defective all of a sudden due to mutation during early development. For example : Albinism (non-heritable).

A gene mutation may alter the synthesis or activity of an enzyme needed for the normal completion of chemical reactions or for the normal functioning of an organism.

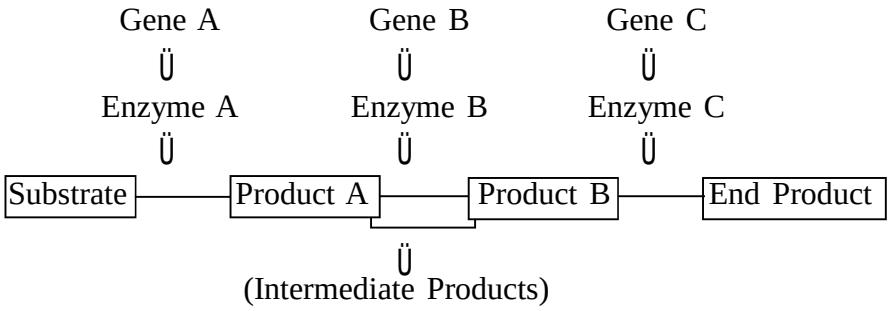
The consequences are :

(i) accumulation of the metabolic substances that are toxic, or

(ii) deficiency of a compound that is important for normal cell functioning.

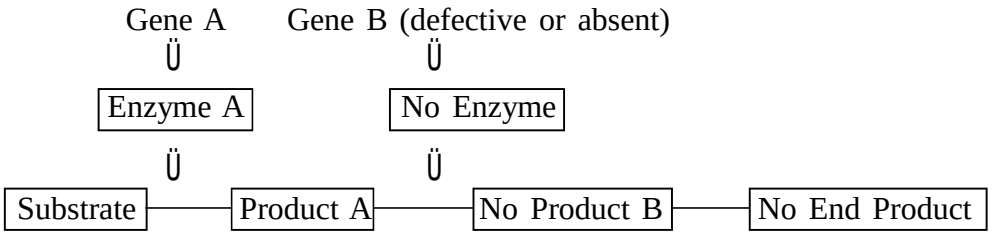
There are mutations that can lead to disorders in any part of the body, including muscles, eyes, liver, bones, kidneys, nerves, blood system etc. Under normal conditions, genes work in total harmony completing their specific job of converting a raw material to a finished product, by synthesising specific enzyme.

Normal gene functioning



Defective Gene Functioning

Sometimes, absence of a gene or defect in a single gene may result in defective metabolism and a desired product may never be formed, or there will be a harmful collection of certain material.



This results in accumulation of unuitilised product A and end produces is not produced.

You will be concerned to note that a number of human disorders are caused due to single gene defects., Following table 29.5 gives an idea of some such diseases, the missing or defective gene products and the symptoms.

Table 29.5 Some Common Single Gene Defects

Disease	Gene Product	Symptoms
(i) Severe Combined Immuno Deficiency syndrome (SCID)	Absence of adenosine deaminase	Loss of immunity, T lymphocytes and B lymphocytes in low count.
(ii) Haemophilia	Absence of blood clotting factor VIII	Defective blood clotting, chronic bleeding in joints.
(iii) Sickle Cell anaemia	defective β chain of haemoglobin	damage to heart, spleen, kidney, liver and brain
(iv) Phenylketonuria (PKU)	Accumulation of aminoacid phenyl alanine in blood	Severe mental .retardation, albinism (lack of pigmentation)

29.9.2 Gene therapy

Most of the genetic disorders may result in serious complications, health problems and untimely death. If somehow defective genes could be manipulated to remove such ailments, it will be a great boon to the human society. Attempts are being made

to develop techniques that can replace the defective genes, or enhance their action. Word therapy means treatment and a way to treat defective genes can be called **Gene therapy.** Human gene therapy can be defined in a number of ways.

Gene Therapy is a technique in which a patient (sufferer) is given healthy genes to replace the defective ones inherited from the parents, or to enhance the action/ reaction of the genes they already have.

Gene therapy

Replacement and alteration of defective gene is called Gene therapy.

Human gene therapy (previously known as human genetic engineering) in a broad sense is the addition of functional normal gene or genes to the genetic material contained in the human cell. This is with the aim of correcting an inherited defect.

The ultimate goal is to let 'protein assembling unit' of the cell make desired proteins needed for the normal functioning of an individual. It is like supplying a patient with the necessary gene product formed within the cells by the patient's own body.

Gene therapy in a way regulates and restores the normal gene expression in response to the needs of the affected person. So far there had been no treatment for the genetic defects which can only be corrected by the gene treatment or the gene therapy.

INTEXT QUESTIONS 29.6

1. What causes the alteration of normal functioning of a gene ?

   .....

2. Name two single gene disorders in human beings.

   .....

3. State which cells have a low count in Severe Combined Immuno Deficiency.

   .....

4. Define gene therapy.

   .....

29.9.3 Approach to human gene therapy

There are two basic approaches to human gene therapy:

(i) Somatic gene therapy, and

(ii) Germ-line gene therapy.

(i) Somatic (body cell) gene therapy

Once a normal gene has been cloned, it can be used to correct a genetic defect. Body cells are targeted for genetic transformation (defective gene transformed to normal). This approach helps in the correction of a genetic defect confined to a specific organ or tissue.

(ii) Germ line (sex cell) gene therapy

In this approach, cells of germinal epithelium or gametes or zygote are genetically modified to create an individual that will carry remedial gene(s) in the following generation. Presently all research on human gene therapy is directed towards correcting gene defects in somatic cells (non-sex cells). Somatic gene therapy can be grouped under the broad categories of :

(a) Ex-vivo gene therapy,

(b) In-vivo gene therapy, and

(c) Antisense gene therapy.

(a) Ex-vivo (outside the body) gene therapy:

This type of therapy usually involves the use of cells (with defective gene) taken from the patient. After the gene alteration when the same cells are transfused (transferred back), no immunological response takes place. The steps involved in the procedure are :

1. Isolating cells with gene defects from a patient.
2. Growing the isolated cells in culture.
3. Altering the isolated cells with remedial gene.
4. Selecting, growing and testing the altered cells.
5. Transplanting or transfusing the altered cells back into the patient (Fig. 29.6).

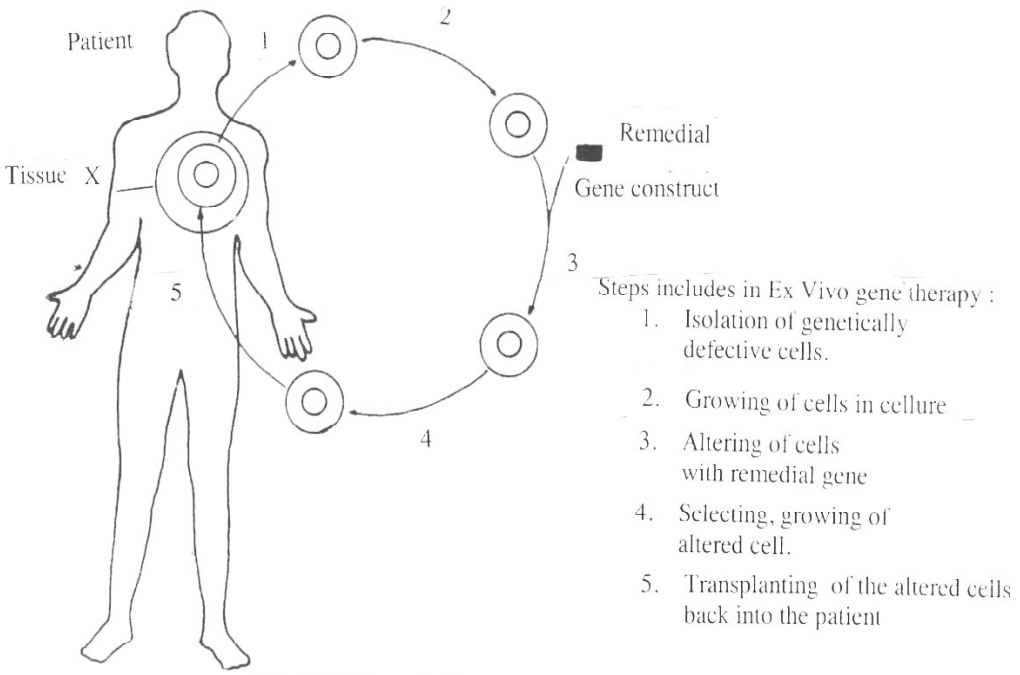


Fig. 29.6 Steps in the Ex-vivo gene therapy

Vectors such as retrovirus are used for the integration of normal gene in the host gene. Stem cells of the bone marrow are continuously producing new cells. If such cells are taken and put back after alteration, to remove genetic defects, these cells

can divide and differentiate into various important cells such as B cells and T cells, macrophages, red blood cells, platelets and bone cells.

Genetically engineered stem cells on transplanting back into the patient's body result in a continuous supply of the required gene product. The technique can be used in the treatment of the following genetic disorders:

(i) Severe Combined Immuno Deficiency (SCID).

(ii) Sickle cell anaemia.

(iii) Thalassemia

(iv) Certain tumours.

(b) In-vivo (within the body) gene therapy

This type of gene therapy includes direct delivery of a remedial gene into the cells of a particular tissue of the patient. Adenovirus, double stranded DNA virus, is being used as a vehicle for transferring the remedial gene, (Fig. 29.7). The viruses used are weak enough to cause any disease. These tissue specific virus integrates with the host genome and can only infect dividing cells and not the other healthy cells.

This therapy may become useful in the treatment of cancer, Alzheimer's disease and Parkinsons's disease.

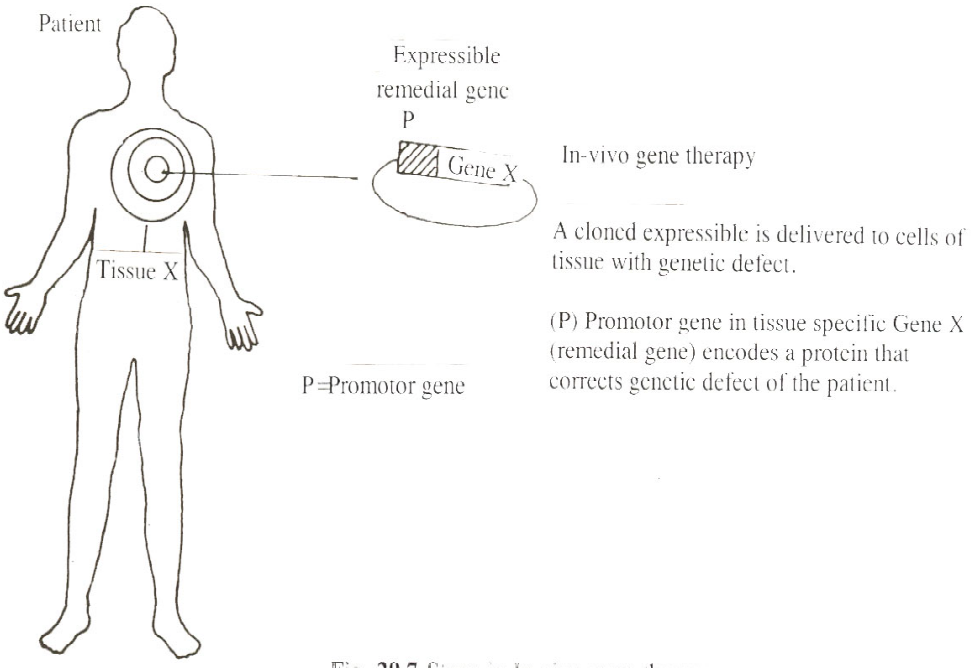


Fig. 29.7 Steps in In-vivo gene therapy

(c) Antisense Therapy

You have learnt the steps involved in protein synthesis - which are transcription and translation.

This therapy is designed to prevent or lower the expression of specific gene thus limiting the amount of translation of protein from the over producing gene.

This therapy involves the introduction of nucleic acid sequence that is complementary to all or part of m-RNA (messenger RNA formed in the target cell) into the cells overproducing the gene product (Fig. 29.8). This therapy will prove useful in certain human genetic diseases and cancers where too much of a gene product or its continuous presence changes the normal functioning of the cell. It has been tried for treatment of malignant glioma or brain tumour. Flavr-savr tomato with a long shelf life has been produced by this technique.

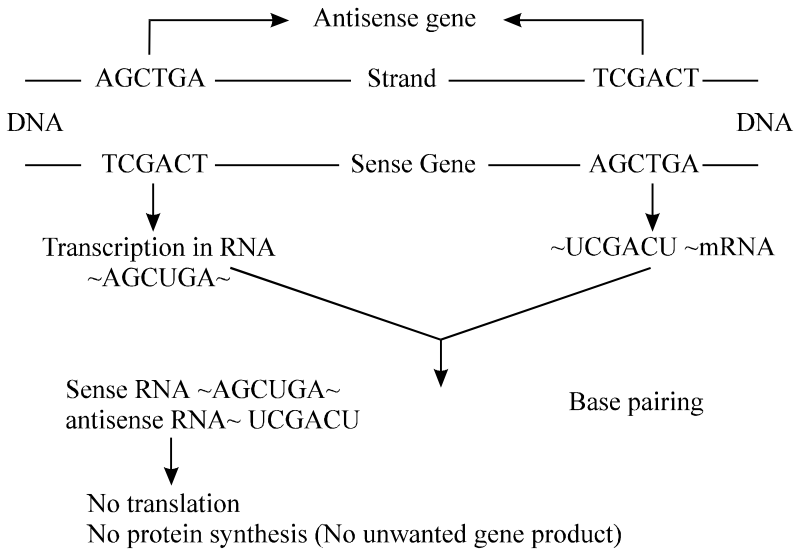


Fig. 29.8 Antisense gene therapy

Antisense gene therapy

An expressible gene is cloned in its reverse (order) orientation and is introduced into a cell. The RNA thus transcribed forms the antisense sequence of normal mRNA. When the antisense RNA base pairs with the mRNA, translation of the mRNA is prevented. The antisense RNA does not contain signals for the initiation of translation.

29.9.4 Gene Therapy—How Far?

The possibility of being able to genetically engineer humans has always been the aim of certain researchers. Somatic cell gene therapy is in its early stages of becoming a mode of treatment for a number of genetic and other diseases such as

(i) AIDS

(ii) Haemophilia

(iii) Atherosclerosis

(iv) Leukaemia

(v) Lung cancer

(vi) Severe Combined Immuno Deficiency-SCID

Germ line gene therapy is not being currently practised. Any manipulation in the genetic material of sex cells may introduce unforeseen characters with alarming

consequences in the offspring. Gene therapy is an expensive and time consuming therapy available only in few advanced countries.

Gene therapy has the following limitations

(i) Research is limited to only somatic cells. Treated individuals can not pass the genetic improvement to offspring.

(ii) There could be a possibility of random integration of DNA into a human chromosome leading to inactivation or activation of a normal gene. This may result in either deficiency of an important enzyme or uncontrolled cell division leading to cancerous growth.

(iii) Planned procedure has to meet strict safety standards in animal trials.

(iv) Target diseases have to be limited to those that involve known defects in a single gene, and the normal gene must be cloned and be available for transplant.

INTEXT QUESTIONS 29.7

1. State the two approaches to human gene therapy.

   .....

2. Name the three categories of somatic cell gene therapy.

   (a) ..... (b) ..... (c) .....

3. Name any two genetic diseases that can be treated by somatic gene therapy.

   (i) ..... (ii) .....

4. What is the direct delivery of the corrected gene into the tissue of the patient by the use of Adenovirus called?

   .....

WHAT YOU HAVE LEARNT

- Biotechnology is the application of scientific knowledge by industries that produce biological products like food supplements, enzymes, drugs etc.
- Yeasts (Fungi), moulds (Fungi) and bacteria are important microorganisms used in industries.
- Yoghurt, alcoholic beverages, antibiotics, vaccines and biogas can be obtained on a commercial scale by the use of microorganisms.
- Fermentation is a process by which sugar is converted into alcohol and CO_2 by bacteria and yeast.
- Fermentation by the yeast *Saccharomyces* yields beer and butter milk.

- In fermentation on large scale, bioreactor and nutrient medium are sterilised by autoclaving. Yeast is inoculated into the medium by support growth system or suspended growth system.
- Yoghurt is made of milk set by a bacterium *Lactobacillus.* Rennet tablets made from calf stomach or ficin from sap of fig trees are used for setting milk into curd.
- Bacteria also yield antibiotics as was discovered by Alexander Fleming. Waksman gave the term antibiotic.
- An antibiotic attacks and terminates a vital step in the metabolic pathway of the pathogenic bacterium which then stops growing.
- Vaccines are prepared (a) from weakened or attenuated germs (first generation vaccines), (b) by recombinant DNA technology (second generation vaccines), or (c) synthetically (third generation vaccines).
- Vitamins may also be generated through fermentation.
- Biogas is made by the action of methanogenic bacteria on waste matter such as the faeces of humans or of cattle.
- Genetic engineering is defined as construction and use of DNA molecules engineered by recombinant DNA technology.
- Recombinant DNA (r-DNA) technology resulted from the discovery of (i) plasmids, and (ii) restriction enzymes.
- Tools of r-DNA technology are cell culture, restriction enzymes, plasmids, ligase and host bacteria.
- Recombinant DNA technology may be used to obtain commercially proteins such as insulin, clotting factors, monoclonal antibodies, enzymes, antibodies and vaccines, etc.
- Genetically engineered organisms carrying foreign genes are called transgenics.
- Transgenic plants may be obtained by using the T_1 plasmid of the bacterium *Agrobacterium tumefaciens*.
- Transgenic animals are produced by microinjection of foreign DNA into fertilised eggs or by using retrovirus for introducing foreign DNA into early embryonic stages.
- Genetically engineered bacteria can clean up pollutants from environment. This is called bioremediation.
- A mutated gene in a cell may result in some form of genetic disorder/disease. Sickle cell anaemia, Haemophilia, SCID etc. are some single gene human disorders.
- Addition of a normal functioning gene to the defective cells to correct the genetic disease is called gene therapy.
- Treatment which is applied to body cells excluding germ line cells is called somatic gene therapy.

- There are three main therapeutic approaches to gene therapy : (a) ex-vivo gene therapy, (b) in-vivo gene therapy, and (c) antisense gene therapy.
- Ex-vivo gene therapy includes addition of corrected genes through retroviral cloning vectors.
- In-vivo gene therapy includes direct delivery of corrected genes into the tissues by use of adenovirus.
- Antisense therapy is designed to prevent or lower the expression of gene in order to have less accumulation of a gene product.
- Gene therapy has certain limitations such as (i) somatic cell gene therapy can not rectify subsequent generation, (ii) random integration of DNA from outside may interfere with normal gene, (iii) strict safety standards are to be maintained, (iv) proper clones of requisite genes have to be available.

TERMINAL EXERCISES

1. Define biotechnology.
2. How are alcoholic beverages produced by fermentation? Mention the steps in the process.
3. How can you make cheese and curd on a large scale?
4. What are antibiotics? Name five antibiotics and their sources.
5. How are different generations of vaccines produced?
6. Describe the steps in the production of biogas and mention the precautions to be taken.
7. Enumerate in a sequence the steps in recombinant DNA technology.
8. Describe the uses of genetic engineering.
9. How can a transgenic animal be obtained?
10. Write a note on bioremediation.
11. Define the term gene therapy. Under what condition does it become necessary to opt for such a therapy ?
12. What is meant by human somatic gene therapy? How does it differ from the germ line gene therapy? Which of the two has been successful so far and why?
13. Discuss in brief the different types of somatic gene therapy.

ANSWERS TO INTEXT QUESTIONS

29.1 1. Fungi, yeast, bacteria

2. Alcohol/antibiotics/curd/cheese/vitamins/vaccines/biogas (any three)
3. Ethanol/Butanol/Glycerol (any two)

4. Support growth system and suspended growth system
5. 1-b, 2-c, 3-a

29.2 1. *Lactobacillus*
2. Alexander Fleming
3. Vaccines produced by the use of recombinant DNA technology
4. Vitamin C
5. Methanogenic bacteria

29.3 1. Construction and use of novel DNA molecules obtained by recombinant DNA technology.
2. Clone is a collection of genetically identical cells obtained by asexual division of a cell.
3. When a fragment of foreign DNA is inserted in DNA of a phage or plasmid, DNA of the latter is called r-DNA.
4. In bacteria
5. Because they can cut specific sequences of DNA. (6) Ligase.
7. A phage or plamid which can carry foreign DNA and divide along with the bacterium whose part it is.

29.4 1. (i) Insulin, Growth hormone (ii) Proteases, Amylases
2. Antibiotics, vaccines and proteins of clinical value can be manufactured abundantly.
3. Rabies and hepatitis B

29.5 1. An organism containing foreign DNA in its genome
2. *Agrobacterium tumefaciens* and T_1 plasmid.
3. Bioremediation is removal of pollutants in the environment with the use of genetically engineered bacteria.

29.6 1. Mutation
2. Haemophilia, Sickle cell anaemia, SCID (Any two)
3. B-cells and T-cells
4. Replacement and alteration fo defective gene is called gene therapy.

29.7 1. Somatic and Germ line cells
2. In-vivo gene therapy, Ex-vivo gene therapy and Anti-sense gene therapy
3. Thalassemia, certain types of cancer
4. In-vivo gene therapy