RECOMBINANT DNA TECHNOLOGY AND BIOTECHNOLOGY

Introduction to Biotechnology and Recombinant DNATechnology

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Keywords

Recombinant biotechnology; Medical biotechnology; Gene therapy; DNA fingerprinting; Vaccines; Animal biotechnology; Xenobiotics; Bioremediation; Tissue culture; Genetic engineering; Transgenic plants; Genetically modified crops; Marine biotechnology; Industrial biotechnology; Fermenter technology; Enzyme technology.

"Sometimes people ask me what field I'd be in if not computers, I think I'd be working in biotechnology. I expect to see breathtaking advances in medicine, over the next two decades and biotechnology researches and companies will be at the center of that progress."

Bill gates
New York Times, June 18, 1996

Introduction to Biotechnology

The term biotechnology is a fusion of biology and technology. The area is multidisciplinary, vast and highly divergent, which has made a precise definition of the subject rather difficult. It is basically the controlled use of biological agents, such as micro organisms or cellular components for human beneficial use. It is the integrated use of biochemistry, microbiology and engineering sciences in order to exploit microorganisms, cultured tissues/cells, to their best. Man has continued his quest for improving the natural capabilities of micro organisms and making them capable of novel processes and to create them for highly valuable cause, for human welfare. Years ago, people exploited micro-organisms for making bread, brewing alcohol and cheese production, although the phenomenon of fermentation was not understood thoroughly. Now, the extent of biotechnological application is more sophisticated. Researchers can manipulate living organisms and transfer genetic material between organisms, generating transgenics (plants/animals). The current applications of biotechnology are predominantly practiced in the field of agriculture and medicine. Modern techniques allow production of new and improved foods. Insect resistant crops have been developed using recent advances in biotechnology. In the field of medicine, it has resulted in development of newer antibiotics, vaccines for various diseases such as cancer, AIDS, hereditary diseases such as Huntington's chorea etc. Biotechnology is also being applied in the area of pollution control, mining and energy production (biofuel production). Genetically engineered micro-organism and plants are used to clean up toxic wastes from industrial effluents and oil spills. It has also found applications in forestry and aquaculture industries. Overall, biotechnology has significantly impacted and improved quality of life and there are many exciting opportunities in biotechnology sector.

Introduction to Recombinant Biotechnology

The spectacular progress and enormous understanding over the past two decades in biological processes at both molecular and cellular level is revolutionized by the advent of recombinant DNA technology or Genetic engineering. This field of science is broadly spawned under modern biotechnology, which is precisely the usage of living organisms to produce improved and valuable products for human consumption. Biotechnology is truly multidisciplinary in nature and it encompasses several disciplines of basic sciences and engineering. The science disciplines which are included under biotechnology are:

- Microbiology
- Chemistry
- Biochemistry
- Genetics
- Molecular biology
- Immunology
- Cell and tissue culture
- Physiology

On the engineering side, it leans heavily on chemical and biochemical engineering since large scale cultivation of microorganisms and cells, their down stream processing etc. are based on them.

Development of recombinant biotechnology date back to 1953, when double helical structure of DNA was elucidated by Watson and crick and the genetic code was cracked by Nirenberg. Cohen and Boyer in 1973 invented the technique to cut and paste DNA sequences i.e. the concept of restriction enzymes came into the picture. Since then recombinant DNA technology has rapidly progressed and expanded. It has sparked a new age in disparate fields. It is the benediction of recombinant DNA technology, that now it is possible to put two genes together, to clone the genes for polypeptides like human insulin, growth factors, hormones, interferons, blood clotting factors and viral coat proteins (for vaccines) in bacteria in a way that protein can be expressed and the resulting recombinant protein can be extracted from the cell cultures. Regions of DNA called genes were found to contain information that would lead to synthesis of specific proteins, which are strings of amino acids. Each of the protein is unique in context of its function and the reaction it catalyses. If now one is able to express a natural gene from any organism in a very simple bacterium such as *Escherichia coli*, a bacterium living in intestines that has become the model organism for biotechnology and brought a turning point in the field. Now, one can induce this bacterium to make a lot of protein that is coded by the gene regardless of the nature and source of donor organism. The techniques used include:

- Gene isolation that codes for a particular specific protein
- Cloning of this gene into an appropriate production host
- Improvement of production yields via improving expression by using better promoters, tighter and controlled regulation
- Optimization of media and growth conditions at fermentor scale.

In 1977, the first human protein (somatostatin) was produced in *E. coli* and in 1982; first recombinant protein (human insulin) was released in the markets. In 1985, Kary Mullis conceived the idea of polymerase chain reaction (PCR), which has given recombinant DNA technology a new face and uplift. Molecular ecology, biomolecular archaeology and DNA forensics and fingerprinting are new disciplines that have become possible as a direct consequence of invention of PCR. These all techniques together constitute recombinant DNA techniques, which will be discussed in detail at some length in the chapter.

Thus, the commercial implications of recombinant DNA technology are that large number of proteins that exist in minute amounts in nature can be mass-produced, if required. Moreover, the yields of the desired products can be increased with improved efficiency from nanogram levels to milligram levels. More recent advances in mid eighties and early nineties have made possible to transform even distantly related DNA in another organism i.e. to genetically modify any organism for production of some desired proteins. Such genetically modified organisms (GMOs) are called Transgenics. Transgenics animals and plants, including cows, sheep, tomatoes, tobacco, potatoes, and cotton have now been obtained. The genes so introduced may make the organism more resistant to disease, may influence the rate of fruit ripening or may increase the productivity. This approach helped in release of such GMOs on food and horticulture industries and also into environment.

DNA sequencing can also be performed with increased efficiency and celerity; culminating in success of massive genome sequencing projects, including the completion of human genome project in 2000. Now over 40 million gene sequences are in GenBank, and genome

sequences of hundreds of prokaryotes and dozens of eukaryotes are finished. This has provided an aid in development of in depth and precise knowledge to researchers and molecular biologists in gene structure, function and regulation and hence the effect of their consequential aberrations that can lead to various chromosomal disorders and cancer. Now it is also possible to alter genes *in vitro* to produce modified enzymes with increase stability or different reaction kinetics that can be for goods and services to food processing industries. Researchers are now exploiting recombinant DNA technology based techniques as an art in science to modify and use genes to enhance the productivity for beneficiary aspects.

Subfields of Biotechnology

1. **Red Biotechnology** – applies to Medical biotechnology, designing of organisms to produce antibiotics and to cure diseases through genetic engineering and manipulations.
2. **White Biotechnology** – (also known as grey biotechnology) is applied to industrial biotechnology
3. **Green Biotechnology** – is biotechnology applied to agricultural processes. This aims at production of more environment friendly solutions than conventional traditional industrial biotechnology.
4. **Bioinformatics** – addresses biological problems with the aid of computational techniques.
5. **Blue Biotechnology** – describes marine and aquatic applications of biotechnology.

Medical Biotechnology

Biotechnology in medicine and pharmacology has been developed in the following areas:-

1. Therapeutics
2. Vaccines, antibodies and drugs
3. New methods of drug delivery
4. Molecular diagnostics
5. Molecular Markers

Product	Uses
• Monoclonal Antibodies produced by hybridoma technology • DNA probes produced by genetically engineered microbes • Recombinant vaccines • Drugs like human insulin, human interferon, human growth hormones • Gene therapy to cure genetic diseases, like Huntington's chorea • *In vitro* fertilization and embryo Transfer techniques, test tube babies, hormone induced super ovulation in cattles • Identification of paternity, criminals using DNA fingerprinting	Used for diagnosis of various diseases. Also used for disease diagnosis Developed Transgenic animals resistant to certain diseases and to produce valuable biochemical that can be excreted in milk urine/ blood, form where they can be extracted and purified.

Biotechnology and Health

Gene therapy has important implications in treatment of acquired and genetic diseases, cancer and possibly AIDS. It is classified into two types:

Germ line gene therapy: where germ cells (sperm or eggs) are modified by the introduction of functional genes. Therefore, the change is heritable and will be passed on to the later generations. This is theoretically highly effective in treating genetic disorders but this option is not considered at present for application in human beings for a variety of ethical reasons.

Somatic gene therapy: where the gene is introduced only in somatic cells but it is not herited as germline is not involved. Somatic gene therapy is further divided into two groups:

The first one where the functional gene is introduced in addition to the defective gene endogenously that is the modified cell contains both the defective as well as the normal (introduced) copies of the gene. This is called as augmentation therapy. The second is targeted gene transfer, which uses homologous recombination to replace the endogenous gene with the introduced functional gene.

The first step in gene therapy is:

- To transform the cell with a specific gene
- Introducing the gene into specific cells within the body (*in vivo*)
- Removing cells from the body
- Introducing the gene and then returning the cells (*ex vivo*).

The in *vivo* therapy involve, use of a vector that carries the gene. The most common types of vectors are viral vectors. Usually the integration of gene into the genome is random and is only transient and is quite possible that indispensable genes may be inactivated or oncogenes may be activated during this phenomenon. Non viral systems of gene delivery are safer comparatively and it includes liposome mediated delivery, electroporation, microinjection etc. Moreover, they do not possess the risk of immune response and are able to survive transport though the body to reach the target cell. Nucleic acid probes can be used to detect variety of plant and animal diseases even before the onset of symptoms. The nucleic acid sequences of pathogen labeled with some markers can be used as probes. Monoclonal antibodies act as an extremely useful tool for rapid and accurate detection and diagnosis of diseases. The advent of hybridoma technology provided methods for the production of specific antibodies targeted against a unique epitope of the immunizing antigen.

DNA Fingerprinting and Forensics

The chemical structure of everyone's DNA is the same. The basic difference between two individual's DNA is the order of base pairs. Using these sequences, every person could be identified solely by the sequence of their base pairs. Scientists usually use a small numbers of sequences of DNA that are known to very among individuals. In medicine, DNA finger printing has application in genetic counselling, proof of parentage, identification of criminals in thefts etc. Since a person inherits his or her VNTRs [variable numbers of tandem repeats, which are, dispersed islands throughout the genome and are made up of a variable numbers of end to end duplications of identical or almost identical sequences of 2-80 each. VNTRs are polymorphic due to difference in numbers of repeat units at a given locus or position in a chromosome] from his or her parents. Thus, analysis of VNTR patterns can be used to

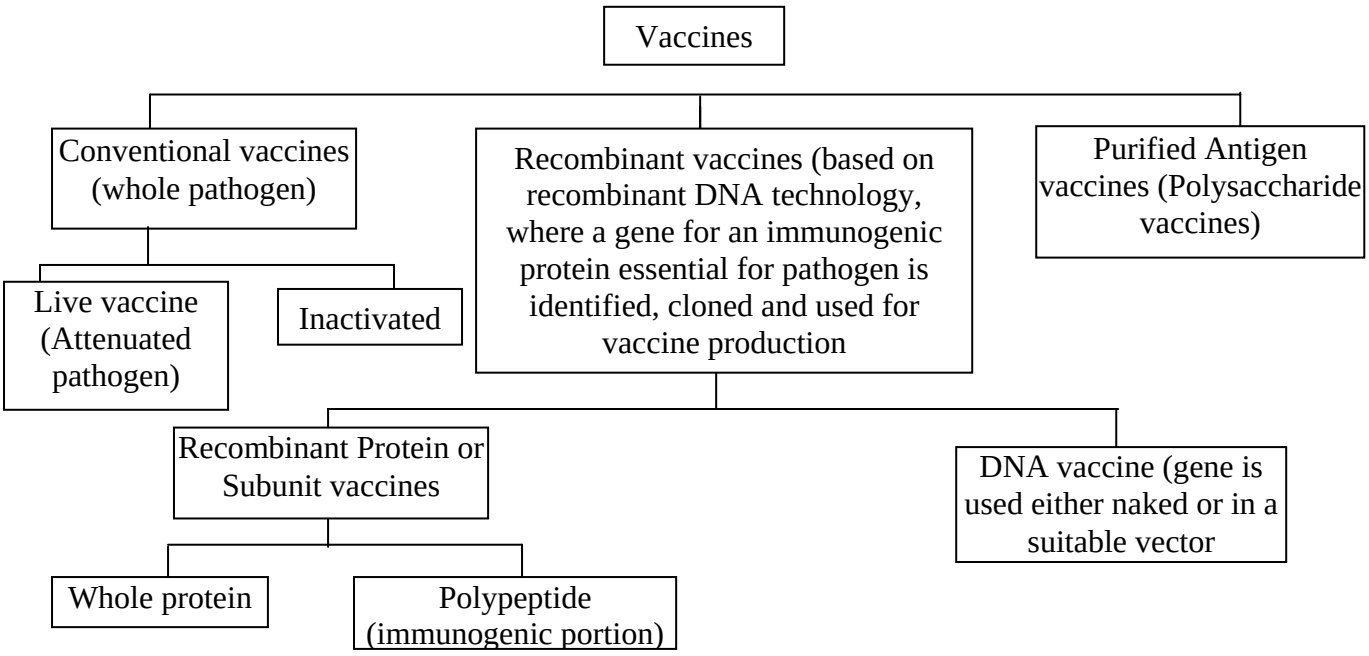
establish paternity and maternity. DNA can be isolated from blood, hair, skin cells etc, and can be compared with that of a suspected criminal for a particular VNTR pattern.

Disease Prevention – Vaccines

Vaccine is the use of biological preparation for immunizations. Vaccines represent an invaluable contribution of biotechnology and provide protection against various diseases. An ideal vaccine formulation should consist of following features:-

- It should not be toxic
- It should be safe with minimum side effects
- It should be eco-friendly
- It should produce long lasting effective humoral and cell mediated immunities.
- It should be simple to administer
- It should be cheap to be affordable to all the classes of people.

Different types of vaccines in commercial use are:-



Conventional vaccines consist of whole pathogenic organisms which are either killed or live but its virulence is greatly reduced (attenuation). It suffers various limitations although it is relatively easy to produce at low cost. It carries a risk of disease due to the occasional presence of active virus particles or reversion of virulence after one round of replication in the vaccinated individuals.

Purified antigen vaccines are based on isolation of antigen from the concerned pathogen. Thus, these non recombinant vaccines do not possess the risk of pathogenicity, since it does not involve whole organism. But the cost is higher due to cumbersome steps involved in purification of antigen and subsequently vaccine preparation.

Many bacteria produce exotoxins, which are highly immunogenic. The toxin, although is inactivated by heat, formaldehyde and other chemicals, most exotoxins, when treated in this way loose their toxicity but still retain its immunogenicity. These are called toxoids and are

used as efficient vaccines. For many pathogenic diseases like tetanus, diphtheria, toxiods are available. Precipitation of toxoids with alum enhances the immunogenicity.

A recombinant vaccine contains either a protein or a gene encoding pathogen's protein that is immunogenic and critical to the pathogen function. The vaccines based on recombinant protein are called as subunit vaccines. The genes encoding such proteins can be identified and isolated form a pathogen and then expressed in *E. coli* or any other host for large scale production of the protein.

Generally, the whole protein molecule is not necessary for immunogenicity, the immunogencity is usually confirmed only by a small portion of the protein molecule. Segments containing these immunogenic residues are effective in immunization and can provide immunity against the deadly pathogen.

Recombinant protein or polypeptide vaccines are safe since whole organisms are not involved. They are highly efficacious. But the cost is high and transportation may pose a problem since protein has to be stored at low temperatures, as heat can destabilize the protein. Thus, their storage and transportation to remote areas nay be problematic and a liming factor in their use.

Recently vaccines based on DNA are being developed. The gene encoding the relevant immunogenic protein is isolated, cloned and then integrated into a suitable expression vector. This is introduced, into the individuals to be immunized. This can generate both humoral and cell-mediated response. Usually the DNA is injected intramuscularly which leads to its uptake and expression of DNA in the muscle cells. Another approach is the use of vectors like vaccinia, adenoviruses, etc for gene delivery. Another approach is to remove cells from the body of an individual into which the concerned immunogen, encoding gene is introduced and expressed. These cells are again introduced into the body.

Disease Diagnosis

An accurate diagnosis of the diseases is critical for its effective management and cure. Following are some ways of disease diagnosis:

1. Microscopy
2. Culturing of the specimen on specific and selective media to allow only specific pathogens to grow, which are then tested for their susceptibility to various therapeutic agents, eg, antibiotics.
3. Immunological detection for the presence of specific antigens on the cell surface of pathogens
4. Detection and quantitation of pathogen – specific antibodies produced in response to the invasion by pathogen.

Novel disease diagnostic approaches have been developed by biotechnology which are efficient, specific precise and rapid.

(a) Use of probes
(b) Use of monoclonal antibodies.

Probes

Probes are small (15-30 bases long) nucleotide (DNA/RNA) sequences used to detect the presence of complementary sequences in nucleic acid samples. The probes may be DNA/RNA or either radioactively or non-radioactively labeled. Use of probes for disease diagnosis is advantageous over conventional diagnostic tools like in the following ways:-

1. High specificity, rapid and much simpler
2. No culturing is required therefore is applicable to those pathogens also which cannot be cultured.
3. Can detect infections even in a very latent stage where antibodies are yet not generated.
4. Probe can be easily prepared
5. A single species-species probe can identify all the serotypes of pathogen.

Monoclonal antibodies

A monoclonal antibody is specific to a single antigenic determinant (epitope) of a single antigen. Usually monoclonal antibodies are produced from hybridomas, where each clone is a product of fusion of a single myeloma cell with a single antibody producing lymphocyte. Monoclonal antibodies are currently employed for classification of blood groups, clear, early and specific detection of pathogens. The immunological assay generally employed for diagnostic of diseases is ELISA. Thus, their high specificity makes them powerful diagnostic tools and also as therapeutic compounds–e.g. delivering toxins to a cancer cell while avoiding healthy cells, [immunotoxins].

A large number of human genes encoding pharmaceutically valuable proteins have been cloned and expressed in microorganisms. Usually *E. coli*, was used as the host for obvious reasons of ease in handling and cloning procedures. Nowadays yeast is becoming the host of choice for production of recombinant proteins. Few of them are shown below:-

Product	**Genetically engineered Micro-organism**	**Application**
Insulin	*E. coli* /yeast	Diabetes
Human growth hormone	*E. coli*	Dwarfism
Interferon	*E. coli*	Viral diseases
Hepatitis B surface antigen	Yeast	Vaccine against hepatitis- B
Streptokinase and urokinase	*E. coli*	Thrombosis
Epidermal growth factor	*E. coli*	Wound healing
Bovine growth hormone	*E. coli*	Increased milk yield
Tumor necrosis factor	*E. coli*	Antitumor and antiviral therapy
Hemoglobin	*E. coli*	Blood substitute
Atrial natriuretic Factor (ANF)	Yeast	Hypertension and Kidney diseases
Nerve growth Factor	*E.coli* Yeast	Peripheral neuropathies

Animal Biotechnology

Animal cell cultures have been used to generate valuable products based on their own genetic information or due to genes transferred to them (Transgenes) using recombinant DNA technology. Transgenic animals have been produced by two methods:

1. Microinjection of cloned genes into the pronucleus of a fertilized ovum.
2. Injection of embryonic stem cells into embryos.

The first method is the most widely and commonly used for producing transgenic mice. After microinjection, the fertilized single cell embryos are removed from the animal. Then the foreign DNA is injected into the embryo's pronucleus. After the injection, the embryos are transferred back into the hormonally prepared or pseudo pregnant recipient females. The second method involves microinjection of embryonic stem cells derived from the inner cell mass of blastocyst stage embryos into embryos to produce two or more distinct cell types. The Embryonic cells are able to produce all tissues of an individual. Once isolated, these embryonic cells are grown in unlimited numbers which are capable of developing into fully formed adults. These cells may then be altered genetically before being used to produce embryos. When these transformed cells participate in the formation of sperm and eggs, transgenic offspring will be produced.

The various objectives for which transgenics are produced are listed below:

1. Gene transferred and expressed into cultured cell line to obtain a biochemical product.
2. Genetic modification of recipient to improve the quality of product produced.
3. Large scale production of the proteins encoded by these genes in milk, urine or blood. This approach is called Molecular Farming or Gene Farming.
4. To introduce functional copies of the defective gene in patients to cure genetic diseases (Gene Therapy).

A transgene must be integrated into the host genome for obtaining transgenic cells/ animals. For this, transgene must be present in proper orientation with various sequences required for its efficient transcription and translation in the host cells. A suitable vector with a promoter, transcription termination sequence, some selective marker for identification and selection of transfected cells should be present.

Some genes transferred and their consequences are mentioned as follows:

Gene transferred	Organism	Applications
Human genes α_1 antitypsin, tissue plasminogen activator, blood clotting factor IX	Cattle, Sheep, Goat, Swine	Genes are expressed in mammary tissues and proteins are secreted in milk in functional form.
Bacterial genes cys E and cys M, concerned with cysteine biosynthesis	Sheep	Improved wool quality
Human hemoglobin and specific antibodies	Mice, Swine	Genes are expressed and proteins released in blood serum, for disease diagnosis
Human growth hormone	Swine, Sheep	Improvement in body weight, feed efficiency, promotes growth and thus meat production.
Salmon growth hormone	Fish	Increased body growth, up to 60% increase in size
Antifreeze protein gene, α-globin gene, *E.coli* hygromycin resistance gene and *E.coli* β-galactosidase gene	Fish	Genes expressed for variable purposes in individuals, where they are inherited stably.

In Vitro Fertilization and Embryo Transfer

Union of egg cell and sperm outside the body in a culture vessel is known as *in vitro* fertilization. This involves collection of healthy ova and sperms from healthy females and males, and their fusion under *in vitro* conditions. The resulting zygote may be cultured *in vitro* for a period of time, which is then implanted in the uterus of healthy female. This technique of *in vitro* fertilization and embryo transfer are done to obtain desirable genotypes and in cases of infertility. *In vitro* fertilized embryos at 16 celled stage have been successfully transferred into the uterus. The babies produced using this approach is termed as Test tube babies. The first test tube baby, named Loise joy Brown, was born on 25th July, 1978. However, this has few ethical and social issues related which may need resolution. Although high degree of expertise is required and the cost of production of each progeny is more, the gains will be attractive and it will be possible to obtain relatively rare genotype.

Environmental Biotechnology

Extensive use of pesticides and industrialization in agriculture, pollution of the environment with man-made (synthetic) organic compounds has become a major problem. Usually, the biodegradable compounds will be broken down and assimilated by various micro organisms but non-biodegradable, recalcitrant compounds may be toxic and hazardous. Such non-biodegradable, man made compounds introduced into nature are called as ***Xenobiotics*** which are not degraded easily by natural microbial flora, eg DDT, BHC, organophosphates. They remain in nature for several years in the toxic form. Biodegradability or recalcitrant depends on the nature of the chemical molecule. ***Biomagnification*** is a phenomenon of progressive increase in the concentration of a Xenobiotic compound as it passes through the food chain.

Bioremediation is a strategy to control pollution with the use of biological system to catalyze the degradation or transformation of various toxic chemicals to less harmful forms.

Biodegradation is the general term used for biologically mediated breakdown of chemical compounds and complete biodegradation leads to mineralization. Fungi are a group of micro-organisms that secrete a variety of intracellular enzymes. They are good in accumulation of heavy metals such as cadmium, copper, mercury; lead etc. *phanerochaete sordida* is useful in the degradation of PAHs (polycyclic aromatic hydrocarbons) from soil. *P. chrysosporium* has been shown to degrade a numbers of toxic xenobiotics such as aromatic hydrocarbons, organochlorines etc. Enzymes like laccases, polyphenol oxidases, lignin peoroxidases play role in degradative process. Yeasts, like *Candida tropicalis, S. cerevisiae* are helpful in clearing industrial effluent. Several bacteria can also degrade toxic pesticides such as halocarbons. Aromatic nitrogen compounds are highly recalcitrant because of the strong aromatic rings. Petroleum products contain a mixture or hydrocarbons, which are difficult to degrade. Pseudomonas species can degrade aromatic compounds like benzene or toluene. Biodegradation of oil spills is a major problem. Moreover, a single bacterium cannot degrade all the components of oil which are petroleum products. ***Anand Chakrabarty***, an Indian scientist, genetically engineered a strain of *Pseudomonas putida* that can degrade more than 3-4 compounds of petroleum. Future, work aims at cloning of highly efficient degradative enzyme producing genes into bacteria for biotechnological aspect. The high surface to cell ratio of fungi makes them better degraders. Thus more research will be focused in future on using the diverse fungal flora for bioremediation. Fungi recently have been shown to even solubilize coal partially, a highly polymeric substance more complex than lignin. Thus, there is an unexplored potential in fungal flora that remained to be harnessed more and more in environmental bioremediation for future.

Plant and Agricultural Biotechnology

With increase in demand for fruits and vegetables, there is an urgent need to integrate biotechnology to speed up the crop improvement programs. Biotechnology tools have revolutionized the entire crop improvement programs by providing new strains of plants, more efficient, specific and selective pesticides and improved fertilizers. Biotechnology has provided new tools and strategies in the developing counties to combat the struggle against food production problem. Ancient people controlled the quantity and quality of the plants they grew, by adopting selective breeding. By collecting seeds from the most desirable plants, they could develop plants which produced more food and were more adapted to their environments. Various methods are adopted for improvement of crops and are as follows:-

1. Tissue culture
2. Genetic engineering

Tissue Culture is one of the most widely used techniques for rapid asexual *in vitro* propagation. The various objectives achieved by plant biotechnology are:

a) Large scale cell cultures for useful biochemical production
b) Rapid clonal multiplication
c) Virus elimination
d) Development of homozygous lines by producing haploids (anther/ ovary culture)
e) Production/ recovery of hybrids – embryo rescue

Continuous biochemical production requires *in vitro* culture of cells for product isolation. For this, rapid clonal multiplication, haploid production, scaling up of culture operations becomes essential. Scaling up procedures involve utilization of fermenters.

Genetic Engineering

It involves three major steps

1. Identification and isolation of suitable genes for transfer.
2. Delivery system for the transfer of desired genes
3. Expression of new genetic information in recipient cells.

Transgenic Plants

When a gene from one species is transferred into another species, the modified organism is called transgenic. Transgenic plants are developed that are resistant to herbicides allowing farmers to spray them so as to kill only weeds but not the crops. Many herbicide tolerant plants have been developed. Transgenic plants resistant to herbicides have been developed by transferring genes that produce enzymes which confer this resistance. Viruses are the major pests of crop plants which causes considerable losses. Many strategies have been applied to control viral infection. Few transgenic resistant plants have been developed against Alfalfa Mosaic Virus, Potato Virus X, Rice Virus, Tobacco rattle virus. Many of the antifungal compounds synthesized by plants which combat fungal infections have been identified. Transgenic plants with antifungal molecules like proteins and toxins have been developed. A number of genes responsible for providing resistance against stresses such as to water stress, heat, cold, salt, heavy metal etc. have been identified. Studies are underway on metabolites like proteins and betaines that have been implicated in stress tolerance. Resistance against chilling was introduced into tobacco plants by introducing gene for glycerol-1-phosphate acyl-transferase enzyme from *Arabidopsis*. Many plants respond to drought stress by synthesizing a group of sugar derivatives called polyols (Mannitol, sorbitol etc.) Plants that have more polyols are more resistant to stress. By transferring a bacterial gene capable of synthesizing mannitols, it is possible to raise the level of mannitol making plants, resistant to drought. Transgenic tomato with reduced pectin methyl estrase activity and increased level of soluble solids and higher pH increases the processing quality. These tomatoes which ripen slowly are helpful in transportation process. Tomatoes with delayed ripening have been produced by knocking down enzymes involved in ethylene production or by using gene for deaminase, which degrades 1-aminocyclopropane-1-carboxylic acid (ACC), an immediate precursor of ethylene. Thus it can increase the shelf life. Tomatoes with elevated sucrose and reduced starch could also be produced using sucrose phosphate synthase gene. Starch content in potatoes has been increased by 20-40% by using a bacterial ADP glucose phosphorylase gene.

The insecticidal beta endotoxin gene (bt) has been isolated from *Bacillus thuringiensis,* the commonly occurring soil bacteria is transferred to a number of plants like cotton, tobacco, tomato, potato etc. to make them insect resistant. These genes produce insecticidal crystal proteins which affect a range of lepidopterans, coleopteran and dipteran insects. These crystals upon ingestion by the insect larva are solublized in the highly alkaline midgut into toxins.

By using recombinant DNA technology, it is possible to genetically manipulate different strains of bacteria suitable to different environmental conditions and to develop strains with traits for better competitiveness. Biopesticides are nothing but biological organisms which can be formulated as pesticides for the control of pests. They are advantageous as they are specific to the target pests and donot harm the non target organisms such as bees, butterflies and are safe to humans and live stocks. They donot harm or disturb the food chain nor leave

behind toxic residues. They degrade rapidly in the environment which is a major environmental benefit. Thus it provides a useful alternative to traditional methods of insect pest control.

Integration of the Transgenes

The major part of DNA introduced into the plant cells is degraded and only a small portion of it becomes integrated into the plant genome. The integration occurs at random sites. Linear DNA is much more effective in producing stable integrations than circular DNA. Multicopy integrations usually occur in tandem at one site. *Agrobacterium* mediated gene transfers leads to integration in the nuclear genome, exceptionally in the chloroplast genome, and that too integration occurs randomly. Only stably integrated transgenes are inherited in Mendelian fashion and in subsequent generations, some instability may occur probably due to methylation or rearrangement.

Other techniques use physical or chemical agents to transfer DNA into plant cells. Protoplasts, are plant cells without their protective cell walls, will take up pure DNA, when treated with certain membrane active agents or with electroporation, giving a rapid high pulsed voltage. Success rates, however, are low, and the techniques are not very reproducible. DNA can also be microinjected into target plant cells using thin glass needles. This is quite laborious and technically difficult. *Biolistics*, a new method, involves very small accelerating particles of tungsten or gold coated with DNA injected into cells using an electrical pulse or air pressures. As the particles pass through the cell, the DNA dissolves and become free to integrate into the plant genome.

Some Important Plant Biotechnologies

1. Gene Transfers (genetic Engineering) for insect resistance, protection against viruses, herbicide resistance, storage protein improvements, cold and saline stress tolerance etc.
2. Molecular markers eg RFLPs and RAPDs for linkage mapping and mapping of quantitative trait loci
3. Germplasm conservation through storage in liquid nitrogen or by slow growth
4. Rapid clonal multiplication through meristem culture

a) Edible Vaccines

Antigens of several pathogens, when delivered orally, produce immunogenic response. Such antigens are good candidates for edible vaccines. For this, the gene encoding the orally active antigenic protein is isolated from the pathogen, and a suitable construct for constitutive or tissue specific expression of the gene is made. The gene construct is introduced and stably integrated into the genome of selected plant species, and is expressed to produce the antigen. The appropriate plant parts containing the antigen may be fed raw to animals or humans to bring about immunization. For animals, crops used as feed eg alfalfa and other forage crops, are suitable for the expression of such antigens, while for humans, fruits like banana, which are consumed in raw form, can be used. The edible vaccines are expected to alleviate the storage problems, offer an easy delivery system by feeding and would have much lower cost than the recombinant vaccines. An example of edible vaccine is provided by the *E. coli* labile enterotoxin, which is expressed in potato. The heat labile toxin causes diarrhea, and is structurally, functionally and antigenically very similar to the cholera toxin.

Transgene integrated	Source	Expressed in	Consequence of Expression and Application
Granule bound starch synthase	Potato	Potato	No amylose synthesis
Mannitol-1 phosphate dehydrogenase	*E. coli* (gene mtl D)	Tobacco	Mannitol at >6 μmol/g fresh wt, increased tolerance to high salinity.
Antibodies (IgG, IgM)	Mouse	Various plants	
Hirudin	Synthetic	*B. napus*	Thrombin inhibitor
α-amylase	*B. licheniformis*		Liquefaction of starch
Phytase	*Aspergillus niger*		Increased phosphate utilization
Xylanase	*C. thermocellum* *C. albidus*		Animal feed, paper and pulp baking.
ADP-Glucose pyro-phosphorylase	*E. coli* (gene glg C16)	Potato	60% more starch than controls.
Cyclodextrin glycosyl transferase	*Klebsiella pneumoniae*	Potato	α and β cyclodextrins are produced
Heat labile enterotoxin B subunit	*E. coli*	Tobacco, potato	Fed orally in mice, comparable to bacteria-derived LT-B

b) Genetically Modified Crops

Genetically Modified Crops are foods that have a gene extracted from a living thing, which has been placed into a different food. This creates plants that nature never could. The purpose for genetic modification is for many different purposes, the main one being to create a food able to survive being sprayed with harmful chemicals like pesticides and herbicides. Other purposes are to make food stay fresher for longer, to kill pests, to produce more of the crop and to experiment with taste and quality.

What are the benefits of Genetic Modification?

- It can increase production and lower the cost of food.
- Gene modification can boost immunity and develop inbuilt vaccines for livestock and poultry.
- Gene technology can remove lactose, so that lactose-intolerant people can eat dairy products.
- Animals which have increased resistance, productivity, hardiness, and feed efficiency can be obtained for better yields of milk, eggs and meat.
- Crops could be grown in areas suffering from drought and salt.
- GM crops are faster and cheaper.
- GM Foods are sometimes thought as being more nutritious, tasting better and they keep longer.
- Many people rely on genetically modified foods for medicines, for example insulin for diabetics.
- GM Foods are safe.

What are the disadvantages of Genetic Modification?

- GM crops can contaminate other crops by pollens from one field to another.
- Sometimes GM crops have allergenic effects.
- Loss of nutritional value.
- Reduction of the efficiency of antibiotics.
- New viruses could evolve from the mass production of GM crops.
- Pests may develop resistance to GM crops that have been designed to kill them.
- GM crops may cause harm to the wealth and welfare of animals.
- Not affordable.
- GM crops may produce ecological side effects.

Thus, genetic modification is till date an issue that whether it is safe to consume GM crops or not.

What crops are produced through Genetic Modification?

1. Golden Rice

Millions of people in the world suffer from Vitamin A deficiency, which leads to vision impairment and increased susceptibility to diarrhea, respiratory diseases, and measles. Rice is a staple food in many countries, particularly in Asia, but traditional varieties of rice does not contain Vitamin A or its immediate precursors. However, geranylgeranyl diphosphate (GGDP), a compound naturally present in immature rice endosperm can be converted to provitamin A with the help of several enzymes, which are not present in rice. Two genes from daffodil and one from the bacterium *Erwinia uredovora* were inserted in the rice genome. These three genes produce the enzymes necessary to convert GGDP to provitamin-A. The inserted genes are controlled by specific promoters such that the enzymes and the provitamin-A are only produced in the rice endosperm. When golden rice is ingested, the human body splits the provitamin-A to make vitamin A. The researchers have successfully produced rice capable of synthesizing beta-carotene, the precursor of Vitamin A. Thus, Golden rice is the result of an effort to develop rice varieties that produce provitamin-A (beta-carotene) as a means of alleviating vitamin A (retinol) deficiencies in the diets of poor and disadvantaged people in developing countries.

2. Tomato

Tomatoes are one of the world's most popular vegetable. Lycopene, a naturally occurring constituent of tomato, is a nutritional factor related to Vitamin A. Tomato varieties with enhanced lycopene content are under experimentation and studies. Another trait of interest is delayed ripening. Tomatoes that ripen slower can remain on the vine longer and develop improved flavor, compared to commercial varieties that are picked at the green stage. The Flavr-Savr® tomato, one of the earliest approved transgenic crop varieties, was a delayed ripening variety. Salty soils are an increasing problem in many parts of the world. Many crop plants, including tomatoes, are killed by high salt levels in soil and irrigation water. The development of a salt-tolerant tomato offers the possibility that tomatoes could be grown on land that was previously unavailable for agriculture. A group of few scientists have developed a tomato plant that is able to tolerate high levels of salt and that holds the salt in its leaves, so the fruit will not taste salty.

3. Canola

Canola is a major oilseed crop. Transgenic research has focused on improving the nutritional quality of canola oil by enhancing the Vitamin E content or by modifying the balance of fatty acids.

4. Sunflower

A disease-resistance trait, an anti-pest trait, and a herbicide-resistance trait are all being pursued, but no commercial varieties is in the market.

5. Coffee and Tea

Decaffeinated coffee is now made by treating coffee beans to remove the caffeine. One method uses organic solvents to extract the caffeine, which is still not very popular, as residues from the solvents will remain in the coffee making it unfit for use. Other methods are criticized for removing some of the desirable, flavor-producing components along with the undesirable caffeine. Scientists have identified different genes that lead to the production of caffeine in coffee beans and tea leaves. If the expression of these genes can be "turned off" in plants, coffee and tea trees could be developed that would produce naturally decaffeinated products with full flavor and aroma.

6. Papaya

Papaya is a tropical fruit rich in Vitamins A and C, but susceptible to a number of serious pests and diseases. The transgenic variety UH Rainbow, resistant to the papaya ringspot virus, is currently in production.

7. Tobacco

Nicotine-free tobacco is now being grown for the production of nicotine-free cigarettes. Previous attempts to make low-nicotine products removed some of the flavor along with the nicotine. Genetically engineered nicotine-free tobacco doesn't synthesize nicotine in the leaf.

8. Trees

Forest trees such as poplar, aspen, and spruce have been transformed with various genes to provide resistance to insects, tolerance to herbicides, and higher levels of the commercial product. For example, reducing the lignin content of a tree can make it easier to recover wood pulp.

poplar	Herbicide tolerance insect resistance
eucalyptus	herbicide tolerance
aspen	reduced lignin
sweetgum	herbicide tolerance
white spruce	insect resistance

Foods nutritionally enhanced through biotechnology, which may be used in the future:

1. Cooking oils with healthier fats to lower cholesterol levels
2. Tomatoes with more lycopene to help prevent cancer
3. Potatoes that absorb less fat during frying to lower cholesterol levels
4. Cereals and vegetables with increased protein content
5. Foods that can protect people from chronic diseases - such as juices and cereals containing extra calcium to reduce osteoporosis

6. Peanuts, milk, and wheat with allergenic proteins removed - allowing people with allergies to eat them
7. Cow's milk that contains extra lysozyme - a natural anti-bacterial compound which could help prevents infection in infants and increases the shelf-life of milk
8. Meats with less fat and better flavor

Marine Biotechnology

Biotechnology in Fisheries and Aquaculture

Marine environment covers approximately 70% of the earth's surface. Since life originated in oceans, it still provides a valuable gene pool. Aquaculture industry is under rapid progress due to increasing demands of fish and shell fish. Marine biotechnology is involved in aquaculture and the isolation of natural products from marine organisms. Algae are important organisms as a source of food and commercial products. Seaweeds are harvested for food and medicinal purposes. Kelp is used for fertilizer and as a source of potash and acetone for the production of explosives. Alginate products are used for their gelling, emulsifying and stabilizing properties. Agar is used in food, pharmaceuticals and culture of micro-organisms. Some marine organisms produce many metabolites, which control pests, some may have antiviral characteristics, and some may treat various cancers.

The fisheries and aquaculture industries in Asia contribute about 45 percent of world fish production. These industries are significant contributors to the food supply, livelihood, foreign exchange earnings The ability to produce transgenic fish and shellfish in culture, which grow faster and larger with more efficient utilization of nutrients, is of particular value to developing countries, not only as a source of food, but also as export products. Biotechnology offers great promises for fish disease control and feed production as well. The injection of pituitary gland extracts into mature fishes for the induction of spawning has been widely practiced in Asia during the past two decades. The use of purified gonadotropin hormone synthetic luteinizing hormone-releasing hormone (LH-RH) has further improved induced spawning techniques. As regards genetic engineering, Chinese scientists have succeeded in identifying and isolating the gene controlling growth hormone (GH) from salmon, common carp, grass carp and silver carp. Transgenic carps carrying human growth hormone gene have been produced. Antifreeze gene has also been identified and used for improving the antifreeze ability of Tilapia. To supplement the fish feed resources, the use of bacterial cells as larval fish feed and processed aquatic plants as grow-out feed has been tested with success in India. Gynogenesis, artificial sex reversal and induced polyploidy are being routinely used for increasing productivity as well as the quality of fish. The growth hormone of salmon and yellowtail is being mass produced through transgenic bacteria and used as a growth promoter on other fish such as rainbow trout. Other useful genes are being identified and introduced into commonly used fish species. New vaccines are also being developed to treat fish diseases.

Besides fish, marine algae are now recognized as important resources for the production of valuable chemicals. Some of the species studied and their products include: *Gracilaria changii* for agar and *Turbinaria conoides, Sargassum baccularia and Sargassum siliquosum* for alginic acid and antimicrobial compounds.

Biotechnology to Protect Biodiversity

Biotechnology already assists the conservation of plant and animal genetic resources through:

- new methods for collecting and storing genes (as seed and tissue culture)
- detection and elimination of diseases in gene bank collections
- identification of useful genes
- improved techniques for long-term storage
- Safer and more efficient distribution of germplasm to users

Tissue culture, which involves growing small pieces of plant tissue or individual cells in culture, provides a fast and efficient way of taking numerous cuttings from a single plant. In many cases, entire plants can be regenerated from a single cell because each cell contains all the necessary genetic information. After selecting a disease-free cutting, for example, scientists can mass-produce copies that are genetically identical. This is the basis of plant cloning, or micropropagation of plants. In gene banks, tissue culture is now used routinely to preserve the genetic information of plants which have seeds that do not store well, are sterile or have poor germination rates. Plant cells maintained on a growth medium in a test-tube replace seeds or plants.

Industrial Biotechnology

'Never under-estimate the power of the microbe'

W.Foster 1964

Industrial biotechnology involves:

- Production of useful compounds eg. Ethanol, lactic acid, glycerine , citric acid , gluconic acid , acetone by microorganisms from less useful substrates
- Production of antibiotics like penicillin, streptomycin. Erythromycin, mitomycin, cycloheximide by fungi, bacteria and actinomycetes as secondary metabolites)
- Fuel produced from cheap, less useful and abundant substrates like Sugarcane biogases, wood etc.
- By the use of enzymatic hydrolysis, pure cellulose can be degraded to soluble sugars which can be fermented to form ethanol, butanol, methane and many other products.
- Organic waste, called biomass, can be converted by microorganisms into alternative fuels, is called bioconversion.
- Mineral Extraction through leaching from low grade ores, eg, copper, uranium etc.
- Enzyme engineering is used for the catalysis of extremely specific chemical reactions, for the immobilization of enzymes. Products formed include L-amino acids, high fructose syrup, semi-synthetic penicillins, starch and cellulose hydrolysis, etc.

Product	Uses
• Production of useful compounds like ethanol each lactic acid, citric acid, gluconic acid, glycerine, acetone etc • Production of antibiotics like penicillin, Streptomycin, erythromycin, mitomycin, etc. • Production of enzymes, eg. Amylase lipases, proteases etc. • SCP from bacteria, fungi or algae for human/animal feed as supplements • Fuel (ethanol/biogas) from cheap, abundant source like sugarcane, wood etc. • Mineral extraction through leaching from low grade ores • Protein/ Enzyme /Antibody engineering	Produced by micro organisms as secondary metabolites Produced by micro organisms Used in detergent, textile, leather and dairy industry SCP is biomass free from any toxins contaminants etc. Produced by fermentation. Microbial action

Microbes have been employed for thousands of years for product generation e.g. wines, bread etc. Following is a table that reflects the contribution of microbes in production of various important products.

S. No.	Microbial Product	Examples
1.	Amino acid	L-glutamic acid, L-Lysine
2.	Antibiotics	Streptomycin, penicillin, tetracycline
3.	Beverages	Wine, beer, distilled beverages
4.	Biodegradable plastic	β-polyhydroxybutyrate (PHB)
5.	Enzymes	Amylase, protease, invertase
6.	Foods	Cheese, pickles, yoghurt, bread, Vinegar
7.	Organic acids	Lactic, citric, butyric, acetic acid
8.	Organic solvents	Acetone, ethanol, butanol
9.	Vitamins	B12, Riboflavin
10.	Recombinant proteins	Insulin, Interferon
11.	Biomass/cell	Organisms used as single cell protein (SCP)
12.	Cells	Biofertilizers, bacterial insecticides, bicontrol agents.

Enzymes are proteins which catalyze specific biochemical reactions efficiently. A number of enzymes which are produced on large scale by microbial action and are used in commercial operations are listed below.

Enzyme	Source	Application
α Amylase	*B.licheniformis, B. amyloliquefaciens*	Hydrolysis of starch to dextrans
Xylose isomerase	*B.coagulans*	Pure glucose to glucose + fructose
Alkaline protease	*B.licheniformis, B.subtilis*	Detergents (protein digestion)
Acid protease (Rennet)	*A.niger*	Milk coagulation and Cheese flavour enhancement
Pectinase	*A.niger, B.subtilis*	Pectin hydrolysis in fruit juices
Lipase	*Rhizopus spp*	Detergents, lipid hydrolysis
Lactase	*A.niger*	Milk lactose hydrolysis to glucose + galactose
Glucanase	*A.niger*	In fruit juices
Invertase	*Saccharomyces*	Used in confectionary
Endogenous protease		Tenderisation of meat and flavour development
Glucose Oxidase		D-glucose oxidized to gluconic acid, O_2 utilized, H_2O_2 produced
Catalase		Degrades H_2O_2 in Water and O_2, used in combination with glucose oxidase to remove glucose and/ or oxygen from foods.

Many enzymes have applications in medicine. The enzymes prepared must be of high purity and without any contamination. Few enzymes which have applications in treatment of diseases and are produced using various microbes are listed as follows:

Enzyme	Action	Application
α Amylase	Starch hydrolysis	Digestive disorders
Asparginase	Asparagine → Aspartate	Leukemia
Collagenase	Collagen hydrolysis	Skin ulcers
Glutaminase	L-glutamine → L-glutamate	Leukemia
Lysozyme	Bacterial cell wall hydrolysis	Antibiotic
Lipase	Lipid hydrolysis	Digestive disorders
Papain	Protein hydrolysis	Deworming
Protease	Protein hydrolysis	Digestive disorders
Streptokinase	Plasminogen → Plasmin	Blood clots
Urokinase	Plasminogen → Plasmin	Blood clots

Aspartame is a dipeptide containing one residue each of L-aspartic acid and methyl ester of L-phenylalanine. It is 180 times sweeter than sucrose and is used as low calorie sweetener.

Biothenol is produced by micro-organisms like *S. cerevisiae* is used most widely as biofuel for transport purposes especially in Brazil and U.S.A. This can serve as petrol replacement since ethanol has many advantages over petrol. It has a much higher latent heat of vaporization than petrol. Ethanol is burnt completely with higher octane number. Ethanol can mixed with petrol, [20% Ethanol: 80% petrol] to make *Gasohol* used widely in USA.

S. cerevisiae ferments hexoses to produce alcohol. Use of cellulose, sugar and starch crops to produce alcohol is in use nowadays. The cellulose is first converted into glucose and other fermentable sugars which are then converted into alcohol using yeast. The potential area where biotechnology can make a notable contribution are development of more efficient organisms for alcoholic fermentation and use of cellulose and hemicellulose for ethanol production. Butanol, is also produced by strictly anaerobic fermentation (by *C.acetobutylicum*) using molasses as substrate. Butanol may also be used as a mixture with petrol as biofuel.

The following steps are involved in industrial biotechnology:

A. culturing the micro-organism in large scale
B. optimization of production
C. process operation
D. product recovery and downstream processing

The first step is the appropriate choice and identification of a biological agent (micro organism /animal cell/ plant cell) capable of producing desired compound. This requires isolation of the organism from an appropriable habitat and its improvement through strain development strategies. This exploits the knowledge of general biology and ecology. What organism to isolate and from where to isolate is an important factor and then to assess its ability to perform the descried functions though various biochemical tests.

Genetically Engineered Microbes (GEMs) are microbes into which genes have been introduced using recombinant DNA technology. GEMs are capable of producing pharmaceutically useful proteins, new metabolites, can degrade non-biological wastes and detoxify toxic wastes.

Once a suitable stain has been selected, isolated and modified, it needs to be cultured or maintained for long periods to be used for various applications, such strains can be used to produce either biomass, if it is the desired product eg, in the case of single cell protein (SCP) or to recover some compounds from the biomass, or the medium. Thus, to obtain this, it is necessary to culture the strain in large scale. The conditions for maximum production of biomass or any desired compound has to be optimized to improve upon the biochemical yields. The culture conditions have to be precisely regulated and if needed, manipulated to fully exploit the intrinsic capabilities of cells. The culmination of all these steps lies in the recovery of the concerned product in a useful from. The efficiency of product recovery is directly reflected in the cost of the product. The process involved in the product recovery and downstream processing should neither be inefficient nor be costly, which prevents the commercial exploitation of the biotechnological process.

Some products of biotechnology have been around for a long time. Yeasts were first used to brew beer and make wine as long ago as 6000 BC. Leavened bread, which is made using yeast, and cheese, made using bacteria, have been common for hundreds of years. Centuries ago people discovered, accidentally, how to make use of biological processes that occur all the time within living cells although the processes could never be understood. They

discovered, for example, that certain micro-organisms like bacteria and moulds would produce vinegar, beer or wine when grown in large vats. This process was called fermentation. Through trial and error, they learned to control these processes and make large quantities of a limited range of products. But, scientists now understand what many of these biological processes are and how they occur. This has allowed them to develop new techniques to alter or copy some of these natural processes and so to make a much wider variety of products. Some, like cheese, are the same as the products made using traditional biotechnology, but the new methods are quicker, cheaper, and more reliable. Others, such as some new pharmaceuticals, could not be made at all using the older methods.

Cheese

1. The milk protein casein curdles because of the presence of lactic acid bacteria or the enzyme rennin or chymosin.
2. Cheese is the curd separated from the liquid portion of milk, called whey.
3. The growth of microorganisms in cheeses is called ripening.
4. Hard cheese is produced by lactic acid bacteria growing in the interior of the curd.
5. Semi soft cheese is ripened by bacteria growing on the surface
6. Soft cheese is ripened by *Penicillium* growing on the surface.

Other Dairy Products

1. Old-fashioned buttermilk was produced by lactic acid bacteria growing during the butter-making process.
2. Commercial buttermilk is made by letting lactic acid bacteria to grow in skimmed milk for 12 hours.
3. Sour cream, yoghurt are produced by lactobacilli, streptococci, or yeasts growing in low-fat milk.

Nondairy Fermentations

1. Sugars in bread dough are fermented by yeast to ethanol and $C0_2$; $C0_2$ causes the bread to rise.
2. Pickles, olives, and soya sauce are the products of microbial fermentations.

Alcoholic Beverages and Vinegar

1. Carbohydrates obtained from grains, potatoes, or molasses are fermented by yeasts to produce ethanol in the production of beer, ale, and distilled spirits.
2. The sugars in fruits such as grapes are fermented by yeasts to produce wines.
3. In wine-making, lactic acid bacteria convert malic acid into lactic acid
4. *Acetobacter* and *Gluconobacter* oxidize ethanol in wine to acetic acid (vinegar).

Ever since the antibiotic penicillin was discovered, biotechnology has played a key role in the treatment of human diseases. Some antibiotics, like penicillin, are produced naturally by micro-organisms. These can be produced in commercial quantities using traditional fermentation techniques. However, many of the antibiotics we require are not produced in exactly the form we need. Initially scientists had to wait for natural mutations which can accidentally produce the ideal version, or use chemical synthesis techniques to modify the natural product. But now, by using genetic engineering techniques, scientists can alter

existing microorganisms to produce large quantities of antibiotics with desired chemical structures. Antibiotics may function over a wide range of microorganisms and are termed 'broad spectrum', for example chloramphenicol and the tetracyclines which can control such unrelated organisms as the rickettsiae, chalamydiae, and mycoplasma. In contrast, streptomycin and penicillin are examples of narrow spectrum antibodies being effective against only a few bacterial species. Thus, in medicine, biotechnology will have an increasing importance in the production of new and improved products that will contribute to the well-being of mankind.

Fermenter Technology

For each biotechnological process the most suitable containment system must be designed and then monitored and controlled. The environment in which the desired biocatalysts can interact with the environment and material supply is known as the Fermenter. Fermenters range from simple stirred tanks to complex integrated systems involving varying levels of computer input. Fermenters occur in two distinct types. The first are non-aseptic systems where it is not necessary to operate with strictly pure cultures of microorganisms. The other type of fermenter is used for production of such compounds as antibiotics, amino acids, polysaccharides and SCP. The object of any fermenter is to optimize the growth of the organism or of a product produced by the organism. To obtain such conditions, the following must be taken into consideration:

1. An energy source, other essential nutrients to satisfy the needs of the organism,
2. Lack of inhibiting compounds in the medium
3. A reliable inoculum
4. The most advantageous physicochemical conditions.

There are two types of fermentation systems: **closed or open**. A closed system implies that all the nutrient components are added at the beginning of the fermentation process and, as a result, the growth rate of the contained organisms will eventually proceed to zero either due to diminishing nutrients or accumulation of toxic waste products. A modification of the batch process is the fed batch system. Here, volumes of nutrients may be added to augment depletion of nutrients. Overall, the system, however, remains closed and there is no continuous flow. In contrast to the above types, in the open system, organisms and nutrients can continuously enter and leave the fermenter.

To achieve optimization of the fermenter system, the following guidelines must be closely followed:

1. The fermenter should be designed to exclude entrance of contaminating organisms as well as containing the desired organisms
2. The culture volume should remain constant
3. The dissolved oxygen level must be maintained above critical levels of aeration
4. Culture agitation for aerobic organisms
5. Parameters such as temperature and pH must be controlled

Thus the growth of cells on a large scale is called industrial fermentation which is carried out in bioreactors, which control aeration, pH, and temperature. Primary metabolites such as ethanol are formed as the cells grow during logarithmic phase (during the trophophase) while secondary metabolites such as penicillin are produced during the stationary phase (idiophase).

Enzyme Technology

Enzymes are complex organic molecules present in living cells where they act as catalysts in bringing about chemical changes in substances. Although enzymes are only formed in living cells, they can also function *in vitro*. The usage of enzymes in industrial processes to aid in different types of chemical transformations has led to the name enzyme technology. **Immobilization of enzymes** on insoluble polymers, such as membranes and particles, which act as supports or carriers for the enzyme activity, is a new and valuable area of enzyme technology. The enzymes become physically confined during a continuous catalytic process and may be recovered from a reaction mixture and re-used over and over again, thus improving the economy of the process. In this way, it has been found that some enzymes that are rapidly inactivated by heat when in cell-free form can be stabilized by attachment to inert polymeric supports. Whole microbial cells can also be immobilized inside polyacrylamide beads and used for a wide range of catalytic functions. The varied array of new enzymes and whole organism systems those are likely to become available presents exciting possibilities for the future. Present application of immobilized catalysts is mainly confined to industrial processes, for example production of L-amino acids, organic acids and fructose syrup.

These are some of the important areas of biotechnology. There are some very promising results that have been obtained from on going experiments, but there is still a long way to go. Things like insulin and monoclonal antibodies should become available while things like biofuels could still take a few decades.

Few industrial products generated which are for human use are:

1. Most amino acids used in foods and medicine are produced by bacteria.
2. 2. Microbial production of amino acids can be used to produce L-isomers; chemical production results in both D- and L-isomers.
3. Lysine and glutamic acid are produced by *Corynebacterium glutamicum*.
4. Citric acid, used in foods, is produced by *Aspergillus niger*.
5. Enzymes used in manufacturing foods, medicines, and other goods are produced by microbes.
6. Some vitamins used as food supplements are made by microorganisms.
7. Vaccines, antibiotics, and steroids are products of microbial growth.
8. The metabolic activities of *T. ferrooxidans* can be used to recover uranium and copper ores.
9. Yeasts are grown for wine- and bread-making; other microbes (*Rhizobium*, *Bradyrhizobium*, and *Bacillus thuringiensis*) are grown for agricultural use.
10. Various microorganisms are grown for use in different industries. For example, the nitrogen-fixing bacteria, *Nitrosomonas* and *Nitrobacter*, are grown in large batches, lyophilized (freeze-dried), packaged and sold as supplements for their use in aquariums. These microorganisms convert toxic ammonia from fish wastes into nitrite and nitrate, respectively that can be recycled by plants.