1 An Introduction to Biotechnology

Abstract

Biotechnology is multidisciplinary field which has major impact on our lives. The technology is known since years which involves working with cells or cell-derived molecules for various applications. It has wide range of uses and is termed "technology of hope" which impact human health, well being of other life forms and our environment. It has revolutionized diagnostics and therapeutics; however, the major challenges to the human beings have been threats posed by deadly virus infections as avian flu, Chikungunya, Ebola, Influenza A, SARS, West Nile, and the latest Zika virus. Personalized medicine is increasingly recognized in healthcare system. In this chapter, the readers would understand the applications of biotechnology in human health care system. It has also impacted the environment which is loaded by toxic compounds due to human industrialization and urbanization. Bioremediation process utilizes use of natural or recombinant organisms for the cleanup of environmental toxic pollutants. The development of insect and pest resistant crops and herbicide tolerant crops has greatly reduced the environmental load of toxic insecticides and pesticides. The increase in crop productivity for solving world food and feed problem is addressed in agricultural biotechnology. The technological advancements have focused on development of alternate, renewable, and sustainable energy sources for production of biofuels. Marine biotechnology explores the products which can be obtained from aquatic organisms. As with every research area, the field of biotechnology is associated with many ethical issues and unseen fears. These are important in defining laws governing the feasibility and approval for the conduct of particular research.

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1.1 Introduction

The term "biotechnology" was coined by a Hungarian engineer Karl Ereky, in 1919, to refer to the science and methods that permit products to be produced from raw materials with the aid of living organisms. Biotechnology is a diverse field which involves either working with living cells or using molecules derived from them for applications oriented toward human welfare using varied types of tools and technologies. It is an amalgamation of biological science with engineering whereby living organisms or cells or parts are used for production of products and services. The main subfields of biotechnology are medical (red) biotechnology, agricultural (green) biotechnology, industrial (white) biotechnology, marine (blue) biotechnology, food biotechnology, and environmental biotechnology (Fig. 1.1.). In this chapter the readers will understand the potential applications of biotechnology in several fields like production of medicines; diagnostics; therapeutics like monoclonal antibodies, stem cells, and gene therapy; agricultural biotechnology; pollution control (bioremediation); industrial and marine biotechnology; and biomaterials, as well as the ethical and safety issues associated with some of the products.

The biotechnology came into being centuries ago when plants and animals began to be selectively bred and microorganisms were used to make beer, wine, cheese, and bread. However, the field gradually evolved, and presently it is the use or manipulation of living organisms to produce beneficiary substances which may have medical, agricultural, and/or industrial utilization. Conventional biotechnology is referred to as the technique that makes use of living organism for specific purposes as bread/cheese making, whereas modern biotechnology deals with the technique that makes use of cellular molecules like DNA, monoclonal antibodies, biologics, etc. Before we go into technical advances of DNA and thus recombinant DNA technology, let us have the basic understanding about DNA and its function.

The foundation of biotechnology was laid down after the discovery of structure of DNA in

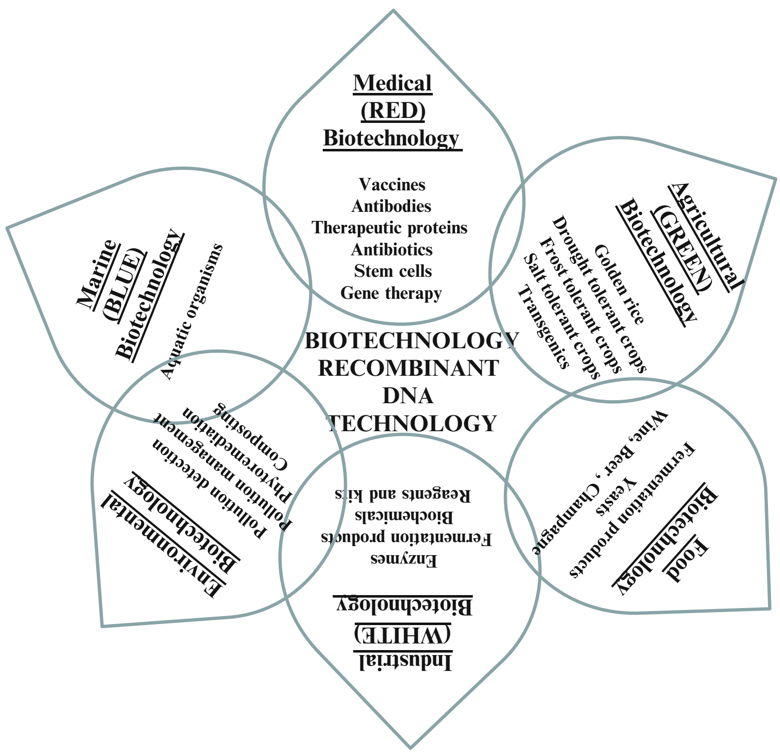

Fig. 1.1 Major applications of biotechnology in different areas and some of their important products

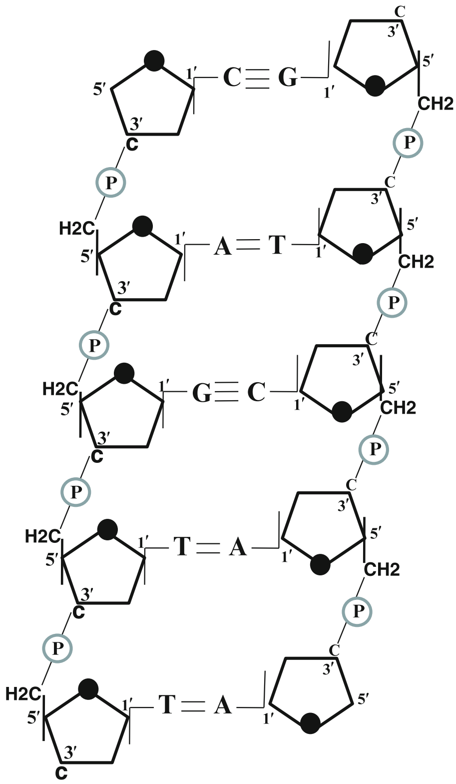

Fig. 1.2 The double helical structure of DNA where both strands are running in opposite direction. Elongation of the chain occurs due to formation of phosphodiester bond between phosphate at 5′ and hydroxyl group of sugar at 3′ of the adjacent sugar of the nucleotide in 5–3′ direction. The sugar is attached to the base. Bases are of four kinds: adenine (*A*), guanine (*G*) (purines), thymine (*T*), and cytosine (*C*) (pyrimidines). Adenine base pairs with two hydrogen bonds with thymine on the opposite antiparallel strand and guanine base pairs with three hydrogen bonds with cytosine present on the opposite antiparallel strand

the early 1950s. The hereditary material is deoxyribonucleic acid (DNA) which contains all the information that dictates each and every step of an individual's life. The DNA consists of deoxyribose sugar, phosphate, and four nitrogenous bases (adenine, guanine, cytosine, and thymine). The base and sugar collectively form nucleoside, while base, sugar, and phosphate form nucleotide (Fig. 1.2). These are arranged in particular orientation on DNA called order or sequence and contain information to express them in the form of protein. DNA has double helical structure, with two strands being complimentary and antiparallel to each other, in which A on one strand base pairs with T and G base pairs with C with two and three bonds, respectively. DNA is the long but compact molecule which is nicely packaged in our nucleus. The DNA is capable of making more copies like itself with the information present in it, as order or sequence of bases. This is called DNA replication. When the cell divides into two, the DNA also replicates and divides equally into two. The process of DNA replication is shown in Fig. 1.3, highlighting important steps.

DNA contains whole information for the working of the cell. The part of the DNA which has information to dictate the biosynthesis of a polypeptide is called a "gene." The arrangement or order of nucleotides determines the kind of proteins which we produce. Each gene is responsible for coding a functional polypeptide. The genes have the information to make a complimentary copy of mRNA. The information of DNA which makes RNA in turn helps cells to incorporate amino acids according to arrangement of letters for making many kinds of proteins. These letters are transcribed into mRNA in the form of triplet codon, where each codon specifies one particular amino acid. The polypeptide is thus made by adding respective amino acids according to the instructions present on RNA. Therefore, the arrangement of four bases (adenine, guanine, cytosine, and thymine) dictates the information to add any of the 20 amino acids to make all the proteins in all the living organisms. Few genes need to be expressed continuously, as their products are required by the cell, and these are known as "constitutive genes." They are responsible for basic housekeeping functions of the cells. However, depending upon the physiological demand and cell's requirement at a particular time, some genes are active and some are inactive, that is, they do not code for any protein. The information contained in the DNA is used to make mRNA in the process of "transcription" (factors shown in Table 1.1). The information of mRNA is used in the process of "translation" for production of protein. Transcription occurs in the nucleus and translation in the cytoplasm of the cell. In translation several initiation factors help in the assembly of mRNA with 40S ribosome and prevent binding of

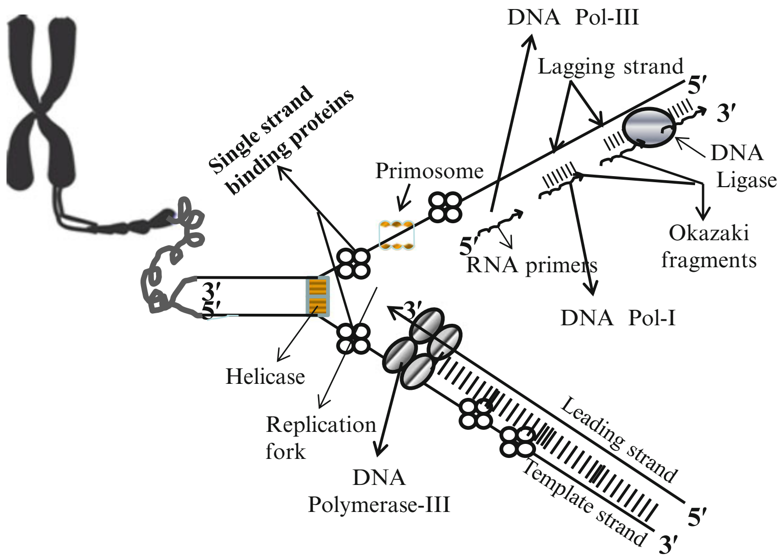

Fig. 1.3 The process of DNA replication. The DNA is densely packed and packaged in the chromosomes. The process requires the action of several factors and enzymes. DNA helicase unwinds the double helix. Topoisomerase relaxes DNA from its super coiled nature. Single-strand binding proteins bind to single-stranded open DNA and prevent its reannealing and maintains strand separation. DNA polymerase is an enzyme which builds a new complimentary DNA strand and has proofreading activity. DNA clamp is a protein which prevents dissociation of DNA polymerase. Primase provides a short RNA sequence for DNA polymerase to begin synthesis. DNA ligase reanneals and joins the Okazaki fragments of the lagging strand. DNA duplication follows semiconservative replication, where each strand serves as template which leads to the production of two complimentary strands. In the newly formed DNA, one strand is old and the other one is new (semiconservative replication). DNA polymerase can extend existing short DNA or RNA strand which is paired to template strand and is called primer. Primer is required as DNA polymerase cannot start the synthesis directly. DNA polymerase is capable of proofreading, that is, correction of wrongly incorporated nucleotide. One strand is replicated continuously with single primer, and it is called as leading strand. Other strand is discontinuous and requires the addition of several primers. The extension is done in the form of short fragments called as Okazaki fragments. The gaps are sealed by DNA ligase. Replication always occurs in 5′–3′ direction

Table 1.1 Factors involved in transcription process

Eukaryotic transcription		
Transcription factor (TF)	Functions	
TFIID	TATA binding	It recognizes
	Protein (TBP)	TATA box
	Subunit	
	TBP associated	Regulate DNA
	Factors	Binding by TBP
TFIIB	Recognizes TFIIB recognition elements (BRE); positions RNA polymerase (RNA pol)	
TFIIF	Stabilizes RNA pol; attracts TFIIE and TFIIF	
TFIIE	Regulates TFIIH	
TFIIH	Unwinds DNA at transcription start point; releases RNA polymerase from promoter	

both ribosomal subunits; they also associate with cap and poly(A) tail. Several elongation factors play an important role in chain elongation. Though each cell of the body has the same genetic makeup, but each is specialized to perform unique functions, the activation and expression of genes is different in each cell. Thus, one type of cells can express some of its genes at one time and may not express the same genes some other time. This is called “temporal regulation” of the gene. In the body different cells express different genes and thus different proteins. For example, liver cell and lymphocyte, would express different genes. This is known as spatial regulation of the gene. Therefore, in the cells of the body, the activation of genes is under spatial regulation (cells present at different locations and different organs produce different proteins) and temporal regulation (same cells produce different proteins at different times). The proteins are formed by the information contained in the DNA and perform a variety of cellular functions. The proteins may be structural (responsible for cell shape and size), or they may be functional like enzymes, signaling intermediates, regulatory proteins, and defense system proteins. However, any kind of genetic defect results in defective protein or alters protein folding which can compromise the functioning of the body and is responsible for the diseases. Figure 1.4 shows the outline of the process of transcription and translation with important steps.

The biotechnological tools are employed toward modification of the gene for gain of function or loss of function of the protein. The technique of removing, adding, or modifying genes in the genome or chromosomes of an organism to bring about the changes in the protein information is called genetic engineering or recombinant DNA technology (Fig. 1.5). DNA recombination made possible the sequencing of the human genome and laid the foundation for the nascent fields of bioinformatics, nanomedicine, and individualized therapy. Multicellular organisms like cows, goats, sheep, rats, corn, potato, and tobacco plants have been genetically engineered to produce substances medically useful to humans. Genetic engineering has revolutionized medicine, enabling mass production of safe, pure, more effective versions of biochemicals that the human body produces naturally [20–22].

The technological advancements have resulted in (1) many biopharmaceuticals and vaccines, (2) new and specific ways to diagnose, (3) increasing the productivity and introduction of quality traits in agricultural crops, (4) the ways to tackle the pollutants efficiently for sustainable environmental practices, (5) helped the forensic experts by DNA fingerprinting and profiling, (6) fermenta-

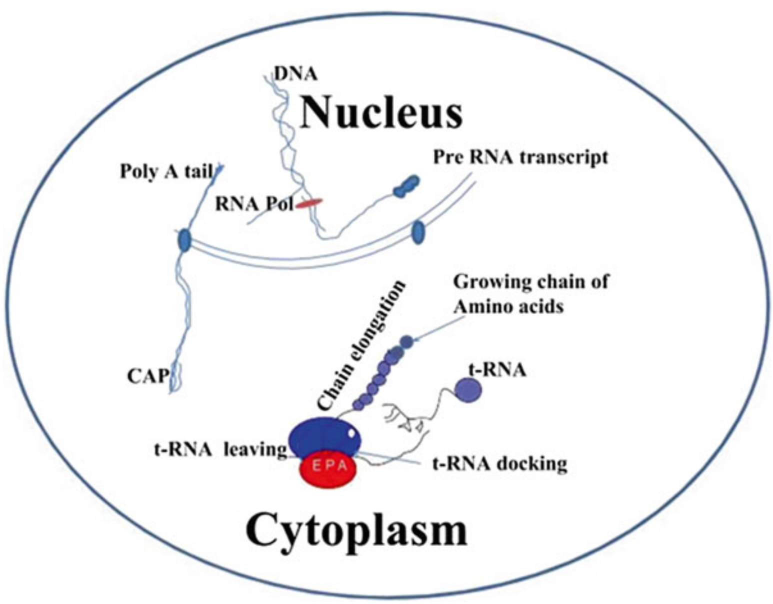

Fig. 1.4 It shows the process of transcription and translation. Transcription occurs in the nucleus and requires the usage of three polymerase enzymes. RNApol I for rRNA, pol II for mRNA, and pol III for both rRNA and tRNA. RNApol II initiates the process by associating itself with seven transcription factors, TFIIA, TFIIB, TFIID, TFIIE, TFIIH, and TFIIJ. After the synthesis, preRNA transcript undergoes processing to form mRNA by removal of introns by splicing and polyadenylation and capping. Protein synthesis is initiated by formation of ribosome and initiator tRNA complex to initiation codon (AUG) of mRNA and involves 11 factors. Chain elongation occurs after sequential addition of amino acids by formation of peptide bonds. Then polypeptide can fold or conjugate itself to other biomolecules and may undergo post-translational modifications as glycosylation or phosphorylation to perform its biological functions

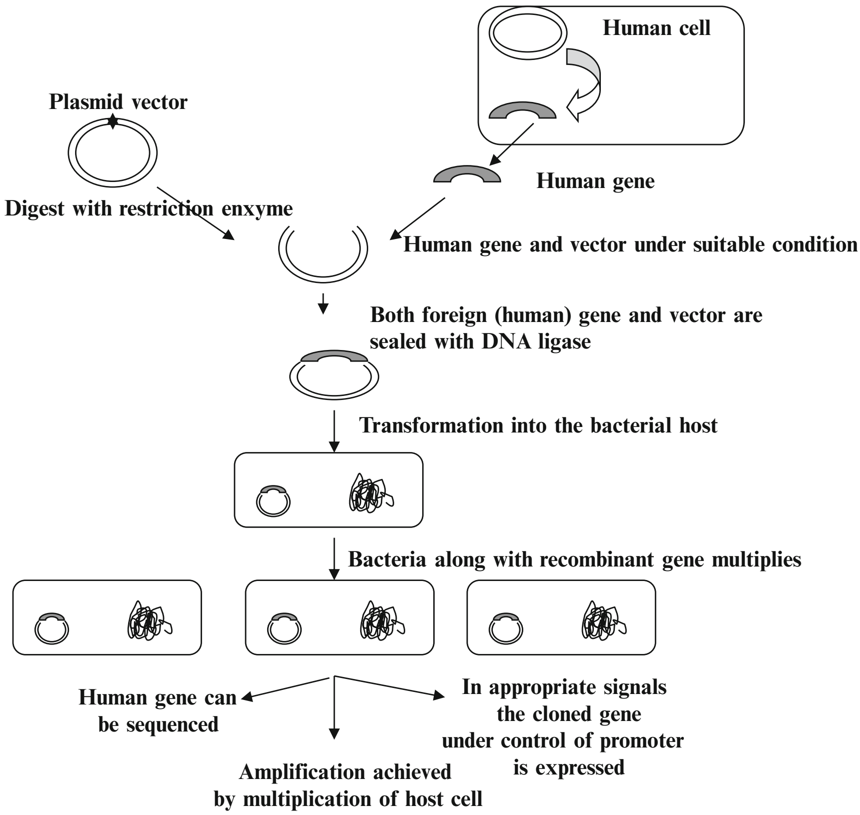

Fig. 1.5 The process of recombinant DNA technology. The gene of interest from human nucleus is isolated and cloned in a plasmid vector. The gene is ligated with the help of DNA ligase. The vector is transformed into a bacterial host. After appropriate selections, the gene is amplified when bacteria multiply or the gene can be sequenced or the gene can be expressed to produce protein

tion technology for production of industrially important products. The list is very long with tremendous advancements and products which have boosted the economy of biotechnology sector worldwide [16]. The biotechnology industry and the products are regulated by various government organizations such as the US Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), and the US Department of Agriculture (USDA).

1.2 Medical Biotechnology

This field of biotechnology has many applications and is involved in production of recombinant pharmaceuticals, tissue engineering products, regenerative medicines such as stem cell and gene therapy, and many more biotechnology products for better human life (Fig. 1.6). Biotechnological tools produce purified bio-therapeutic agents on industrial scales. These include both novel agents and agents formerly available only in small quantities. Crude vaccines were used in antiquity in China, India, and Persia. For example, vaccination with scabs that contained the smallpox virus was a practice in the East for centuries. In 1798 English country doctor Edward Jenner demonstrated that inoculation with pus from sores due to infection by a related cowpox virus could prevent smallpox far less dangerously. It marked the beginning of vaccination. Humans have been benefited incalculably from the implementation of vaccination programs.

Tremendous progress has been made since the early recombinant DNA technology (RDT) experiments from which the lively—and highly profitable—biotechnology industry emerged.

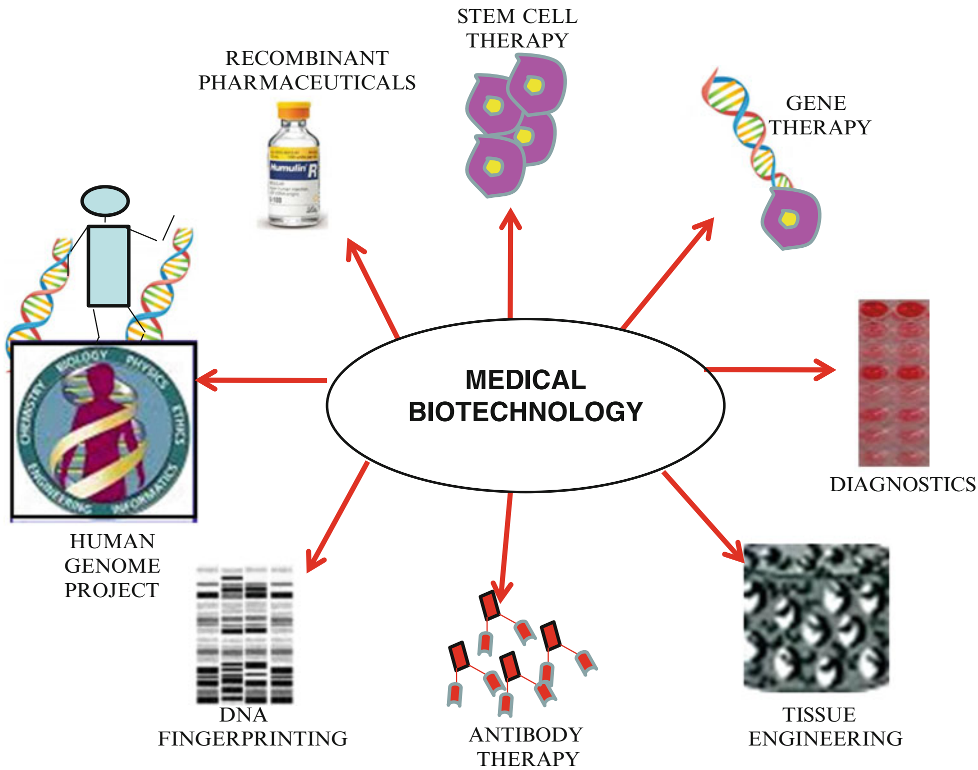

Fig. 1.6 Various applications of medical biotechnology

RDT has fomented multiple revolutions in medicine. Safe and improved drugs, accelerated drug discovery, better diagnostic and quick methods for detecting an infection either active or latent, development of new and safe vaccines, and completely novel classes of therapeutics and other medical applications are added feathers in its cap. The technology has revolutionized understanding of diseases as diverse as cystic fibrosis and cancer. Pharmaceutical biotechnology being one of the important sectors involves using animals or hybrids of tumor cells or leukocytes or cells (prokaryotic, mammalian) to produce safer, more efficacious, and cost-effective versions of conventionally produced biopharmaceuticals. The launch of the new biopharmaceutical or drug requires screening and development. Mice were widely used as research animals for screening. However, in the wake of animal protection, animal cell culture offers accurate and inexpensive source of cells for drug screening and efficacy testing. Pharmaceutical biotechnology's greatest potential lies in gene therapy and stem cell-based therapy. The underlying cause of defect of many inherited diseases is now located and characterized. Gene therapy is the insertion of the functional gene in place of defective gene into cells to prevent, control, or cure disease. It also involves addition of genes for pro-drug or cytokines to eliminate or suppress the growth of the tumors in cancer treatment.

But the progress so far is viewed by many scientists as only a beginning. They believe that, in the not-so-distant future, the refinement of "targeted therapies" should dramatically improve drug safety and efficacy. The development of predictive technologies may lead to a new era in disease prevention, particularly in some of the world's rapidly developing economies. Yet the risks cannot be ignored as new developments and discoveries pose new questions, particularly in areas as gene therapy, the ethics of stem cell research, and the misuse of genomic information.

Many bio-therapeutic agents in clinical use are biotech pharmaceuticals. Insulin was among the earliest recombinant drugs. Canadian physiologists

Frederick Banting and Charles Best discovered and isolated insulin in 1921. In that time many patients diagnosed with diabetes died within a few years. In the mid-1960s, several groups reported synthesizing the hormone.

The first "bioengineered" drug, a recombinant form of human insulin, was approved by the US Food and Drug Administration (FDA) in 1982. Until then, insulin was obtained from a limited supply of beef or pork pancreas tissue. By inserting the human gene for insulin into bacteria, scientists were able to achieve lifesaving insulin production in large quantities. In the near future, patients with diabetes may be able to inhale insulin, eliminating the need for injections. Inhaled insulin products like Exubera® were approved by the USFDA; however, it was pulled out and other products like Technosphere® insulin (Afrezza®) are under investigation. They may provide relief from prandial insulin. Afrezza consists of a pre-meal insulin powder loaded into a cartridge for oral inhalation.

Technosphere technology: The technology allows administration of therapeutics through pulmonary route which otherwise were required to be given as injections. These formulations have broad spectrum of physicochemical characteristics and are prepared with a diverse assortment of drugs with protein or small molecule which may be hydrobhobic or hydrophilic or anionic or cationic in nature. The technology can have its applicability not only through pulmonary route but also for other routes of administration including local lung delivery.

The first recombinant vaccine, approved in 1986, was produced by cloning a gene fragment from the hepatitis B virus into yeast (Merck's Recombivax HB). The fragment was translated by the yeast's genetic machinery into an antigenic protein. This was present on the surface of the virus that stimulates the immune response. This avoided the need to extract the antigen from the serum of people infected with hepatitis B.

The Food and Drug administration (FDA) approved more biotech drugs in 1997 than in the previous several years combined. The FDA has approved many recombinant drugs for human health conditions. These include AIDS, anemia, cancers (Kaposi's sarcoma, leukemia, and colorectal, kidney, and ovarian cancers), certain circulatory problems, certain hereditary disorders (cystic fibrosis, familial hypercholesterolemia, Gaucher's disease, hemophilia A, severe combined immunodeficiency disease, and Turner's syndrome), diabetic foot ulcers, diphtheria, genital warts, hepatitis B, hepatitis C, human growth hormone deficiency, and multiple sclerosis. Today there are more than 100 recombinant drugs and vaccines. Because of their efficiency, safety, and relatively low cost, molecular diagnostic tests and recombinant vaccines may have particular relevance for combating long-standing diseases of developing countries, including leishmaniasis (a tropical infection causing fever and lesions) and malaria.

Stem cell research is very promising and holds tremendous potential to treat neurodegenerative disorders, spinal cord injuries, and other conditions related to organ or tissue loss.

DNA analysis is another powerful technique which compares DNA pattern [14] after performing RFLP and probing it by minisatellite repeat sequence between two or more individuals. Its modification, DNA profiling (process of matching the DNA profiles for STS markers in two or more individuals; see chapter 18), which utilizes multilocus PCR analysis of DNA of suspect and victims, is very powerful and is useful in criminal investigation, paternity disputes, and so many other legal issues. The analysis is very useful in criminal investigations and involves evaluation of DNA from samples of the hair, body fluids, or skin at a crime scene and comparison of these with those obtained from the suspects.

1.2.1 Improved Diagnostic and Therapeutic Capabilities

The sequencing of the human genome in 2003, has given scientists an incredibly rich "parts list" with which to better understand why and how disease happens. It has given added power to gene expression profiling, a method of monitoring expression of thousands of genes simultaneously on a glass slide called a microarray. This technique can predict the aggressiveness of cancer.

The development of monoclonal antibodies in 1975 led to a medical revolution. The body normally produces a wide range of antibodies—the immune system proteins—that defend our body and eliminate microorganisms and other foreign invaders. By fusing antibody-producing cells with myeloma cells, scientists were able to generate antibodies that would, like "magic bullets," bind with specific targets including unique markers, called antigenic determinants (epitopes), on the surfaces of inflammatory cells. When tagged with radioisotopes or other contrast agents, monoclonal antibodies can help in detecting the location of cancer cells, thereby improving the precision of surgery and radiation therapy and showing—within 48 h—whether a tumor is responding to chemotherapy.

The polymerase chain reaction, a method for amplifying tiny bits of DNA first described in the mid-1980s, has been crucial to the development of blood tests that can quickly determine exposure to the human immunodeficiency virus (HIV). Genetic testing currently is available for many rare monogenic disorders, such as hemophilia, Duchenne muscular dystrophy, sickle cell anemia, thalassemia, etc.

Another rapidly developing field is proteomics, which deals with analysis and identification of proteins. The analysis is done by two-dimensional gel electrophoresis of the sample and then performing mass spectrometric analysis for each individual protein. The technique may be helpful in detecting the disease-associated protein in the biological sample. They may indicate early signs of disease, even before symptoms appear. One such marker is C-reactive protein, an indicator of inflammatory changes in blood vessel walls that presage atherosclerosis.

Nanomedicine is a rapidly moving field. Scientists are developing a wide variety of nanoparticles and nanodevices, scarcely a millionth of an inch in diameter, to improve detection of cancer, boost immune responses, repair damaged tissue, and thwart atherosclerosis. The FDA has approved a paclitaxel albumin-stabilized nanoparticle formulation (Abraxane® for injectable suspension, made by Abraxis BioScience) for the treatment of metastatic adenocarcinoma of the pancreas. Nanoparticles are being explored in heart patients in the USA as a way to keep their heart arteries open following angioplasty.

Therapeutic proteins are those, which can replace the patients naturally occurring proteins, when levels of the natural proteins are low or absent due to the disease. High-throughput screening, conducted with sophisticated robotic and computer technologies, enables scientists to test tens of thousands of small molecules in a single day for their ability to bind to or modulate the activity of a "target," such as a receptor for a neurotransmitter in the brain. The goal is to improve the speed and accuracy of therapeutic protein or potential drug discovery while lowering the cost and improving the safety of pharmaceuticals that make it to market.

Many of the molecules utilized for detection also have therapeutic potential too, for example, monoclonal antibodies. The monoclonal antibodies are approved for the treatment of many diseases as cancer, multiple sclerosis, and rheumatoid arthritis. They are currently being tested in patients as potential treatments for asthma, Crohn's disease, and muscular dystrophy. As the antibodies may be efficiently targeted against a particular cell surface marker, thus they are used to deliver a lethal dose of toxic drug to cancer cells, avoiding collateral damage to nearby normal tissues.

1.3 Agricultural Biotechnology

The man has made tremendous progress in crop improvement in terms of yield; still the ultimate production of crop is less than their full genetic potential. There are many reasons like environmental stresses (weather condition as rain, cold, frost), diseases, pests, and many other factors which reduce the ultimate desired yield affecting crop productivity. The efforts are going on to design crops which may be grown irrespective of their season or can be grown in frost or drought conditions for maximum utilization of land, which would ultimately affect crop productivity [24]. Agricultural biotechnology aims to introduce sustainable agricultural practices with best yield potential and minimal adverse effects on environment (Fig. 1.7). For example, combating pests was

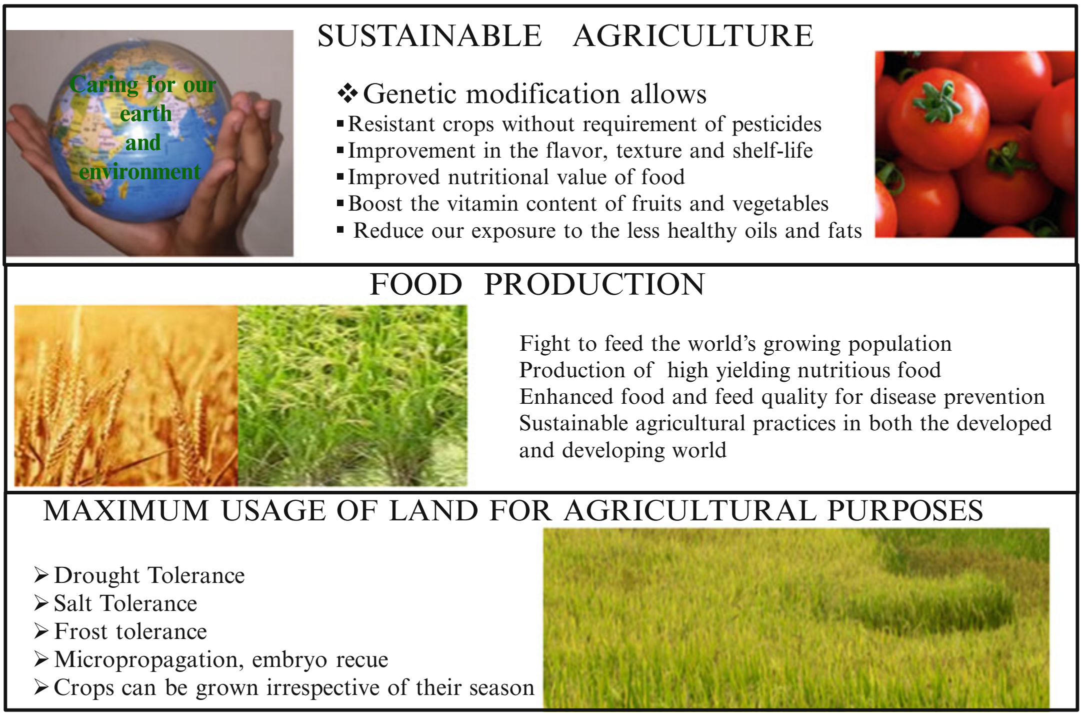

Fig. 1.7 Various applications of agricultural biotechnology

a major challenge. Thus, the gene from bacterium *Bacillus thuringiensis*, the Bt gene, that functions as insect-resistant gene when inserted into crop plants like cotton, corn, and soybean helps prevent the invasion of pathogen, and the tool is called *integrated pest management*. This management is helpful in reducing usage of potentially dangerous pesticides on the crop. Not only the minimal or low usage of pesticides is beneficial for the crop but also the load of the polluting pesticides on environment is greatly reduced [24].

Resistance to Infectious Agents Through Genetic Engineering

(a) Bt crops
- The gene comes from the soil bacterium *Bacillus thuringiensis*.
- The gene produces crystal proteins called Cry proteins. More than 100 different variants of the Bt toxins

(continued)

have been identified which have different specificity to target insect lepidoptera. For eg., CryIa for butterflies and CRYIII for beetles.
- These Cry proteins are toxic to larvae of insects like tobacco budworm, armyworm, and beetles.
- The Cry proteins exist as an inactive protoxins.
- These are converted into active toxin in alkaline pH of the gut upon solubilization when ingested by the insect.
- After the toxin is activated, it binds to the surface of epithelial cells of midgut and creates pores causing swelling and lysis of cells leading to the death of the insect (larva).
- The genes (cry genes) encoding this protein are isolated from the bacterium and incorporated into several

(continued)

crop plants like cotton, tomato, corn, rice, and soybean.

The proteins encoded by the following cry genes control the pest given against them:

- Cry I Ac and cry II Ab control cotton bollworms.
- Cry I Ab controls corn borer.
- Cry III Ab controls Colorado potato beetle.
- Cry III Bb controls corn rootworm.

(b) Protection against nematodes

- A nematode *Meloidogyne incognita* infects tobacco plants and reduces their yield.
- The specific genes (in the form of cDNA) from the parasite are introduced into the plant using *Agrobacterium*-mediated transformation.
- The genes are introduced in such a way that both sense/coding RNA and antisense RNA (complimentary to the sense/coding RNA) are produced.
- Since these two RNAs are complementary, they form a double-stranded RNA (ds RNA).
- This neutralizes the specific RNA of the nematode, by a process called RNA – interference.
- As a result, the parasite cannot multiply in the transgenic host, and the transgenic plant is protected from the pest.

These resistant crops result in reduced application of pesticides. The yield is high without the pathogen infestations and insecticides. This also helps to reduce load of these toxic chemicals in the environment.

The transformation techniques and their applications are being utilized to develop rice, cassava, and tomato, free of viral diseases by "International Laboratory for Tropical Agricultural Biotechnology" (ILTAB). ILTAB in 1995 reported the first transfer of a resistance gene from a wild-type species of rice to a susceptible cultivated rice variety. The transferred gene expressed and imparted resistance to crop-destroying bacterium *Xanthomonas oryzae*. The resistant gene was transferred into susceptible rice varieties that are cultivated on more than 24 million hectares around the world [6].

The recombinant DNA technology reduces the time between the identification of a particular gene to its application for betterment of crops by skipping the labor-intensive and time-consuming conventional breeding [3]. For example, the alteration of known gene in plant for the improvement of yield or tolerance to adverse environmental conditions or resistance to insect in one generation and its inheritance to further generations. Plant cell and tissue culture techniques are one of the applications where virus-free plants can be grown and multiplied irrespective of their season on large scale (micropropogation), raising haploids, or embryo rescue. It also opens an opportunity to cross two manipulated varieties or two incompatible varieties (protoplast culture) for obtaining best variety for cultivation.

With the help of technology, new, improved, and safe agricultural products may emerge which would be helpful for maintaining contamination-free environment. Biotechnology has the potential to produce:

- High crop yields [4]
- Crops are engineered to have desirable nutrients and better taste (e.g., tomatoes and other edible crops with increased levels of vitamin C, vitamin E, and/or beta-carotene protect against the risk of some prevalent chronic diseases and rice with increased iron levels protects against anemia)
- Insect- and disease-resistant plants
- Genetic modification avoids nonselective changes
- Longer shelf life of fruits and vegetables

The potential of biotechnology may contribute to increasing agricultural, food, and feed production, protecting the environment, mitigating pollution, sustaining agricultural practices, and improving human and animal health. Some agricultural crops as corn and marine organisms can be potential alternative for biofuel production. The by-products of the process may be processed to produce other chemical feedstocks for various products. It is estimated that the world's chemical and fuel demand could be supplied by such renewable resources in the first half of the next century [5].

1.3.1 Food Biotechnology

Food biotechnology is an emerging field, which can increase the production of food, improving its nutritional content and improving the taste of the food. The food is safe and beneficial as it needs fewer pesticides and insecticides. The technology aims to produce foods which have more flavors, contain more vitamins and minerals, and absorb less fat when cooked. Food biotechnology may remove allergens and toxic components from foods, for their better utility [6, 7].

1.4 Environmental Biotechnology

Environmental biotechnology grossly deals with maintenance of environment, which is pollution-free, the water is contamination-free, and the atmosphere is free of toxic gases. Thus, it deals with waste treatment, monitoring of environmental changes, and pollution prevention. Bioremediation in which utilization of higher living organisms (plants: phytoremediation) or certain microbial species for decontamination or conversion of harmful products is done is the main application of environmental biotechnology. The enzyme bioreactors are also being developed which would pretreat some industrial and food waste components and allow their removal through the sewage system rather than through solid waste disposal mechanisms. The production of biofuel from waste can solve the fuel crisis (biogas). Microbes may be engineered to produce enzymes required for conversion of plant and vegetable materials into building blocks for biodegradable plastics. In some cases, the by-products of the pollution-fighting microorganisms are themselves useful. For example, methane can be derived from a form of bacteria that degrades sulfur liquor, a waste product of paper manufacturing. This methane thus obtained is used as a fuel or in other industrial processes. Insect- and pest-resistant crops have reduced the use and environmental load of insecticides and pesticides. Insect-protected crops allow for less potential exposure of farmers and groundwater to chemical residues while providing farmers with season-long control.

1.5 Industrial Biotechnology

The utilization of biotechnological tools (bioprocessing) for the manufacturing of biotechnology-derived products (fuels, plastics, enzymes, chemicals, and many more compounds) on industrial scale is industrial biotechnology. The aim is to develop newer industrial manufacturing processes and products, which are economical and better than preexisting ones with minimal environmental impact. In industrial biotechnology, (1) microorganisms are being explored for producing material goods like fermentation products as cheese; (2) biorefineries where oils, sugars, and biomass may be converted into biofuels, bioplastics, and biopolymers; (3) and value-added chemicals from biomass. The utilization of modern techniques can improve the efficiency and reduces the environmental impacts of industrial processes like textile, paper, pulp, and chemical manufacturing. For example, development and usage of biocatalysts, such as enzymes, to synthesize chemicals and development of antibiotics and better tasting liquors and their usage in food industry have provided safe and effective processing for sustainable productions. Biotechnological tools in the textile industry are utilized for the finishing of fabrics and garments. Biotechnology also produces spider silk and biotech-derived cotton that is warmer and stronger and has improved dye uptake and retention, enhanced absorbency, and wrinkle and shrink resistance.

Biofuels may be derived from photosynthetic organisms, which capture solar energy, transform it in other products like carbohydrates and oils, and store them. Different plants can be used for fuel production:

1. Bioethanol can be obtained from sugar (as sugarcane or sugar beet) or starch (like corn or maize). These are fermented to produce ethanol, a liquid fuel commonly used for transportation.
2. Biodiesel can be obtained from natural oils from plants like oil palm, soybean, or algae. They can be burned directly in a diesel engine or a furnace, or blended with petroleum, to produce fuels such as biodiesel.
3. Wood and its by-products can be converted into liquid biofuels, such as methanol or ethanol, or into wood gas. Wood can also be burned as solid fuel, like the irewood.

In these kinds of biological reaction, there are many renewable chemicals of economic importance coproduced as side streams of bioenergy and biofuels as levulinic acid, itaconic acid, and sorbitol. These have tremendous economic potential and their fruitful usage would depend upon the collaboration for research and development between the government and the private sector.

1.5.1 Enzyme Production

The enzymes have big commercial and industrial significance. They have wide applications in food industry, leather industry, pharmaceuticals, chemicals, detergents, and research. In detergents the alkaline protease, subtilisin (from *Bacillus subtilis*), was used by Novo Industries, Denmark. The production of enzymes is an important industrial application with world market of approximately 5 billion dollars. The enzymes can be obtained from animals, plants, or microorganisms. The production from microorganisms is preferred as they are easy to maintain in culture with simple media requirements and easy scale-up. The important enzymes for the industrial applications are in food industry, human application, and research. A few animal enzymes are also important as a group of proteolytic enzymes, for example, plasminogen activators, which act on inactive plasminogen and activate it to plasmin, which destroys fibrin network of blood clot. Some of the plasminogen activators are urokinase and tissue plasminogen activators (t-PA). Urokinase (from urine) is difficult to obtain in ample quantity; thus, t-PA is obtained from cells grown in culture medium. Streptokinase (bacterial enzyme) is also a plasminogen activator but is nonspecific and immunogenic.

Enzyme engineering is also being tried where modifications of specific amino acid residue are done for improving the enzyme properties. One of the enzymes chymosin (rennin) coagulates milk for cheese manufacturing.

The enzymes can be produced by culturing cells, growing them with appropriate substrates in culture conditions. After optimum time the enzymes may be obtained by cell disruption (enzymatic/freeze–thaw/osmotic shock) followed by preparative steps (centrifugation, filtration), purification, and analysis. The product is then packaged and ultimately launched in the market.

After their production, they can be immobilized on large range of materials (agar, cellulose, porous glass, or porous alumina) for subsequent reuse. Some of the important industrial enzymes are α-amylase (used for starch hydrolysis), amyloglucosidase (dextrin hydrolysis), β-galactosidase (lactose hydrolysis), aminoacylase (hydrolysis of acylated L-amino acids), glucose oxidase (oxidation of glucose), and luciferase (bioluminescence). Some of the medically important enzymes are urokinase and t-PA for blood clot removal and L-asparaginase for removal of L-asparagine essential for tumor growth and thus used for cancer chemotherapy in leukemia.

1.5.2 Exploring Algae for Production of Biofuels

The energy requirement of present population is increasing and gradually fossil fuels are rapidly depleting. Thus, renewable energy sources like solar energy and wind-, hydro-, and biomass-based energy are being explored worldwide. One of the feedstocks may be microalgae, which are fast-growing, photosynthetic organisms requiring carbon dioxide, some nutrients, and water for its growth. They produce large amount of lipids and carbohydrates, which can be processed into different biofuels and commercially important coproducts. The production of biofuels using algal biomass is advantageous as they (1) can grow throughout the year and thus their productivity is higher than other oil seed crops, (2) have high tolerance to high carbon dioxide content, (3) utilize less water, (4) do not require herbicides or pesticides with high growth potential (waste water can be utilized for algal cultivation), (5) can sustain harsh atmospheric conditions, and (6) do not interfere with productivity of conventional crops as they do not require agricultural land. The production of various biofuels from algae is schematically represented in Fig. 1.8.

Algae can serve as potential source for biofuel production; however, biomass production is low. The production has certain limitations, as cultivation cost is high with requirement of high energy [1].

1.6 Marine or Aquatic Biotechnology

Marine or aquatic biotechnology also referred to as "blue biotechnology" deals with exploring and utilizing the marine resources of the world. Aquatic or marine life has been intriguing and a source of livelihood for many since years. As major part of earth is acquired by water, thus nearly 75–80 % types of life forms exist in oceans and aquatic systems. It studies the wide diversity found in the structure and physiology of marine organisms. They are unique in their own ways and lack their equivalent on land. These organisms have been explored and utilized for numerous applications as searching new treatment for cancer or exploring other marine resources, because of which the field is gradually gaining momentum and economic opportunities [19]. The global economic benefits are estimated to be very high. The field aims to:

1. Fulfill the increasing food supply needs
2. Identify and isolate important compounds which may benefit health of humans
3. Manipulate the existing traits in sea animals for their improvement
4. Protect marine ecosystem and gain knowledge about the geochemical processes occurring in oceans

Some of the major applications are discussed:

- Aquaculture: Aquaculture refers to the growth of aquatic organisms in culture condition for commercial purposes. These animals may be shellfish, finfish, and many others. Mariculture refers to the cultivation of marine animals. Their main applications are in food, food ingredients, pharmaceuticals, and fuels, the products are in high demand, and various industries are in aquaculture business, for example, crawfish farming (Louisiana), catfish industry (Alabama and Mississippi Delta), and trout farming (Idaho and West Virginia).
- Biotechnology discoveries and products.
  - Transgenic species of salmon with growth hormone gene has accelerated growth of salmons.
  - *Molt-inhibiting hormone* (MIH) from blue crabs leads to soft-shelled crab.
  - *Antifreeze proteins*: A novel protein antifreeze protein (AFP) was identified. AFPs were isolated from Northern cod (bottom-dwelling fish) living at the Eastern Canada coast and teleosts living in extremely cold weather of Antarctica. AFPs have been isolated from *Osmerus mordax* (smelt), *Clupea harengus* (herring), *Pleuronectes americanus* (winter flounder), and many others. Due to antifreeze properties (lowering the minimal freezing temperature by 2–3 °C), the gene has potential for raising plants which are cold tolerant (e.g., tomatoes).
  - *Green fluorescent protein*: A much popular green fluorescent protein (GFP) was obtained from jellyfish *Aequorea victoria*.

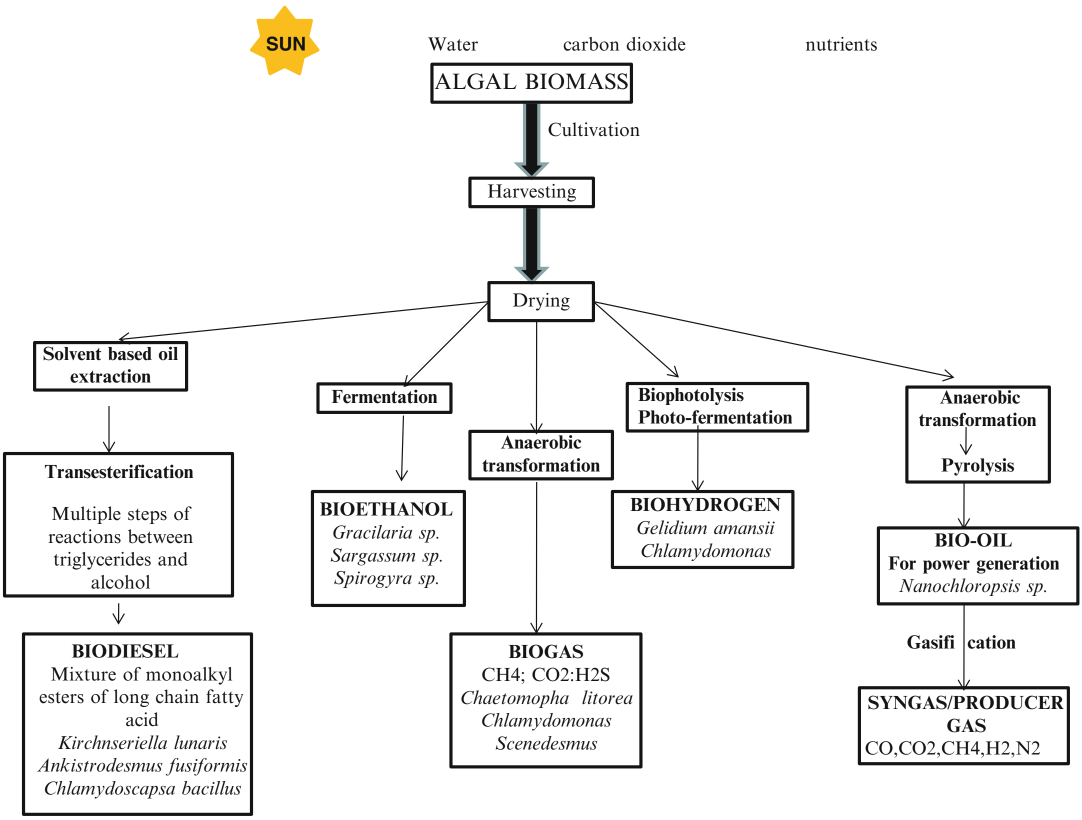

Fig. 1.8 Different biofuel productions by using microalgae. The algae use sunlight, CO_2, water, and some nutrients

It can fluoresce and thus glow in the dark. Many marine microorganisms have bioluminescent capability. GFP is widely used as reporter gene in experiments related to gene cloning, expression, and transgenics. A transgenic strain of zebra fish in the name of GloFish was created by Yorktown Industries, Texas, in 2004. This was with red fluorescent protein gene obtained from sea anemones, and it was the first genetically modified pet animal in the market.

Medicinal applications: For osteoporosis, salmon calcitonin (calcitonin is thyroid hormone promoting calcium uptake and bone calcification) with 20 times higher bioactivity is available as injection and nasal spray.

Hydroxyapatite (*HA*): Obtained from coral reefs and is an important component of bone and cartilage matrix. Its implants are prepared by Interpore Internationals which may be used for filling gaps in fractured bones.

Byssal fibers: Are protein-rich superadhesive which have elastic properties obtained from mussels (*Mytilus edulis*). Their isolation would not be very economical, but they can have wide applications in surgical sutures, artificial tendons, and ligament grafts.

Many anti-inflammatory, analgesic, anticancerous compounds have been identified from sea organisms which can have tremendous potential for human health.

Tetrodotoxin (TTX) is the most toxic poison (10,000 times more lethal than cyanide) produced by Japanese pufferfish or blowfish (*Fugu rubripes*). TTX is being used to study and understand its effect on sodium channels which can help guide the development of drugs with anesthetic and analgesic properties.

Other Products

1. Taq polymerase from *Thermus aquaticus* which is used in PCR reactions and obtained from hot spring Archaea.
2. Collagenase (protease) obtained from *Vibrio* is used in tissue engineering and culturing.

1.7 Transgenic Animals and Plants

In the early 1980s, inserting DNA from humans into mice and other animals became possible. The animals and plants which have foreign gene in each of their cells are referred to as transgenic organisms and the inserted gene as transgene. Expression of human genes in these transgenic animals can be useful in studies, as models for the development of diabetes, atherosclerosis, and Alzheimer's disease. They also can generate large quantities of potentially therapeutic human proteins. Transgenic plants also offer many economic, safe, and practical solutions for production of variety of biopharmaceuticals. The plants have been engineered to produce many blood products (human serum albumin, cytokines), human growth hormone, recombinant antibodies, and subunit vaccines.

The usage of transgenic plants for the production of recombinant pharmaceuticals might open new avenues in biotechnology. As plants can be grown inexpensively with minimal complicated requirements, thus they may have tremendous therapeutic potential. The plants have been engineered to produce more nutrients or better shelf life. The transgenic plants have been created which have genes for insect resistance (Bt cotton, soybean, corn). Now billion acres of land is used for cultivation of genetically engineered crops of cotton, corn, and soybean as they have higher yield and are pest resistant. However, due to social, ethical, and biosafety issues, they have received acceptance as well as rejections at many places and health and environment-related concerns in many parts of the world [8].

1.8 Response to Antibiotic Resistance

Antibiotics are one of the broadly used therapeutic molecules produced by certain classes of microorganisms (bacteria and fungi) which can be used in diverse clinical situations to eliminate bacteria, improve symptoms, and prevent number of infections. Antibiotics have various other applications apart from clinical aspects. They can be used for the treatment of tumors and treatment of meat, in cattles and livestocks, in basic biotechnological work. However, their effectiveness is a matter of concern as bacteria which are continuously exposed to certain antibiotics might become resistant to it due to accumulation of mutations. These days antibiotic-resistant bacteria have increased and some of them have developed multiple drug resistance. Thus, it has become very difficult to initiate therapy in diseases like tuberculosis and leprosy. Biotechnology is solving the urgent and growing problem of antibiotic resistance. With the help of bioinformatics—powerful computer programs capable of analyzing billions of bits of genomic sequence data—scientists are cracking the genetic codes of bacteria and discovering "weak spots" vulnerable to attack by compounds identified via high-throughput screening. This kind of work led in 2000 to the approval of Zyvox (linezolid), an antibiotic to reach the market in 35 years.

Lytic bacteriophage viruses that infect and kill bacteria may be another way to counter resistance. First used to treat infection in the 1920s, "phage therapy" was largely eclipsed by the development of antibiotics. However, researchers in the former Soviet Republic of Georgia reported that a biodegradable polymer impregnated with bacteriophages and the antibiotic Cipro successfully healed wounds infected with a drug-resistant bacterium.

Case Study
After exposure of strontium-90, three Georgian lumberjacks from village Lia had systemic effects, and two of them developed severe local radiation injuries which subsequently became infected with *Staphylococcus aureus*. Upon hospitalization, the patients were treated with various medications, including antibiotics and topical ointments; however, wound healing was only moderately successful, and their *S. aureus* infection could not be eliminated. Approximately 1 month after hospitalization, treatment with PhagoBioDerm (a wound-healing preparation consisting of a biodegradable polymer impregnated with ciprofloxacin and bacteriophages) was initiated. Purulent drainage stopped within 2–7 days. Clinical improvement was associated with rapid (7 days) elimination of the etiologic agent, and a strain of *S. aureus* responsible for infection was resistant to many antibiotics (including ciprofloxacin) but was susceptible to the bacteriophages contained in the PhagoBioDerm preparation [11].

1.9 The Challenges for the Technology

1.9.1 Gene Therapy

Some biotech approaches to better health have proven to be more challenging than others. An example is gene transfer, where the defective gene is replaced with a normally functioning one. The normal gene is delivered to target tissues in most cases by virus that is genetically altered to render it harmless. The first ex vivo gene transfer experiment, conducted in 1990 at the National Institutes of Health (NIH), on Ashanti DeSilva who was suffering from severe combined immunodeficiency (SCID) helped boost her immune response and successfully corrected an enzyme deficiency. However, treatment was required every few months. However, 9 years later, a major setback occurred in gene therapy trial after the death of 18-year-old Jesse Gelsinger suffering from ornithine transcarbamylase (OTC) deficiency due to intense inflammatory responses followed by gene therapy treatment. There were some positive experiences and some setbacks from gene therapy trials leading to stricter safety requirements in clinical trials.

1.9.2 Designer Babies

The fancy term designer baby was invented by media. Many people in society prefer embryos with better traits, intellect, and intelligence. They want to select embryo post germline engineering. This technique is still in infancy but is capable of creating lot of differences in the society thus requires appropriate guidelines.

1.9.3 Genetically Modified Food

Genetically modified crops harboring genes for insect resistance were grown on billion of acres of land. These crops became very popular due to high yield and pest resistance. However, some of the pests gradually developed resistance for a few of these transgenic crops posing resistant pest threat. The other technologies as “traitor” and “terminator” technologies pose serious risk on crop biodiversity and would impart negative characters in the crop (they were not released due to public outcry).

1.9.4 Pharmacogenomics

Scientists do not believe they will find a single gene for every disease. As a result, they are studying relationships between genes and probing populations for variations in the genetic code, called single nucleotide polymorphisms, or SNPs, that may increase one's risk for a particular disease or determine one's response to a given medication. This powerful ability to assign risk and response to genetic variations is fueling the movement toward "individualized medicine." The goal is prevention, earlier diagnosis, and more effective therapy by prescribing interventions that match patients' particular genetic characteristics.

1.9.5 Tissue Engineering

Tissue engineering is one of the emerging fields with tremendous potential to supply replacement tissue and organ option for many diseases. Lot is achieved, lot more need to be achieved.

1.10 Ethical Issues

The pursuit of cutting-edge research "brings us closer to our ultimate goal of eliminating disability and disease through the best care which modern medicine can provide." Understanding of the genetics of heart disease and cancer will aid the development of screening tools and interventions that can help prevent the spread of these devastating disorders into the world's most rapidly developing economies.

Biotechnology is a neutral tool; nevertheless, its capabilities raise troubling ethical questions. Should prospective parents be allowed to "engineer" the physical characteristics of their embryos? Should science tinker with the human germ line, or would that alter in profound and irrevocable ways what it means to be human?

More immediately, shouldn't researchers apply biotechnology—if they can—to eliminate health disparities among racial and ethnic groups? While genetic variation is one of many factors contributing to differences in health outcome (others include environment, socioeconomic status, health-care access, stress, and behavior), the growing ability to mine DNA databases from diverse populations should enable scientists to parse the roles these and other factors play.

Biotechnology along with supportive healthcare infrastructure can solve complicated health problems. Accessibility to the new screening tests, vaccines, and medications and cultural, economic, and political barriers to change must be overcome. Research must include more people from disadvantaged groups, which will require overcoming long-held concerns, some of them have had about medical science.

Biotechnology has been a significant force which has improved the quality of lives and has incalculably benefitted human beings. However, technology does have prospects of doing harm also due to unanticipated consequences. Each technology is subjected to ethical assessment and requires a different ethical approach. Obviously the changes are necessary as technology can have major impact on the world; thus, a righteous approach should be followed. There is uncertainty in predicting consequences, as this powerful technology has potential to manipulate humans themselves. Ethical concerns are even more important as the future of humanity can change which require careful attention and consideration. Therefore, wisdom is required to articulate our responsibilities toward environment, animals, nature, and ourselves for the coming future generations. We need to differentiate what is important technologically rather that what technology can do. For an imperative question, that is, whether this can be achieved, the research must answer "Why should it be achieved"? Who would it benefit?

1.11 Issues Related to Safety

- As the new GM crops are entering the market, the issue regarding their consumption, whether they are safe, without any risk, is one of the important concerns [2]. Though the results related to safety and usage are well reported

(as compared to conventional crops), unknown fear from these products makes them non acceptable at many places [20].
- As insect- and pest-resistant varieties are being prepared and used as Bt genes in corn and cotton crops, there exists a risk of development of resistance insect population. Another important factor is that these resistant crops may harm other species like birds and butterfly.
- The development of more weeds may occur as cross-pollination might result in production of weeds with herbicide resistance which would be difficult to control.
- The gene transfers might cross the natural species boundary and affect biological diversity.
- The judgment of their usage would depend upon the clear understanding of risks associated with safety of these products in determining the impact of these on environment, other crops, and other animal species.

1.12 Future of the Technology

With the understanding of science, we should understand that genetic transfers have been occurring in animals and plant systems; thus, the risk of the biotechnology-derived products is similar as conventional crops [12].

The biotechnology products would be acceptable to many if they are beneficial and safe. People are willing to buy crops free of pesticides and insecticides. Nowadays people are also accepting crops grown without the usage of chemical fertilizers or pesticides, which are high in nutritive values.

The labeling of the product is also an ethical issue as some believe that labeling any product as biotechnology product might be taken by consumer as warning signs; however, others believe that labeling should be done as consumer has every right to know what he is consuming [9]. The products may be acceptable if consumers can accept the food derived from biotechnology weighing all pros and cons and, if the price is right, has more nutritive values, is good in taste, and is safe to consume [10].

Biotechnology is at the crossroads in terms of fears and thus public acceptance [15]. Surprisingly the therapeutic products are all accepted and find major place in biopharmaceutical industry, but food crops are still facing problems in worldwide acceptance. The future of the world food supply depends upon how well scientists, government, and the food industry are able to communicate with consumers about the benefits and safety of the technology [13, 16]. Several major initiatives are under way to strengthen the regulatory process and to communicate more effectively with consumers by conducting educational programs [18, 23].

1.13 Chapter End Summary

- The advantages of biotechnology are so broad that it is finding its place in virtually every industry. It has applications in areas as diverse as pharmaceuticals, diagnostics, textiles, aquaculture, forestry, chemicals, household products, environmental cleanup, food processing, and forensics to name a few.
- Biotechnology is enabling these industries to make new or better products, often with greater speed, efficiency, and flexibility.
- With the applications of recombinant DNA technology, more safer and therapeutic drugs are produced. These recombinant products do not elicit unwanted immunological response which is observed when the product is obtained from other live or dead sources. Many of these therapeutics are approved for human usage, and many of them are in the phase of development.
- Immunological and DNA-based techniques like PCR (polymerase chain reaction) are used for early diagnosis of disorders. PCR and NAAT with microarray can be utilized for the diagnosis of many diseases, and it can detect mutations in gene.
- The technology holds promise through stem cell research and gene therapy and holds applications in forensic medicine.
- The technique may be helpful in developing useful and beneficial plants. It overcomes the limitations of traditional plant breeding. The

techniques of plant tissue culture, transgenics, and marker-assisted selections are largely used for selecting better yielding varieties and imparting quality traits in plants.

- It is also helpful in maintaining environment by bioremediation and other treatment. The areas where it finds applications are:
  - Food industries. Production of single-cell protein, spirulina, enzymes, and solid-state fermentations
  - Increase and improvement of agricultural production
  - Production of therapeutic pharmaceuticals
  - Production of vaccines and monoclonal antibodies
  - Cultivation of virus for vaccine production

Multiple Choice Questions

1. Which abiotic stress can be tolerated by genetically modified crops?
   (a) Insects
   (b) Pests
   (c) Drought
   (d) All of the above
2. The golden rice is a crop having high nutritive value in:
   (a) Vitamin A
   (b) Vitamin B
   (c) Vitamin C
   (d) Vitamin D and calcium
3. Bt toxin gene which encodes cry protein is:
   (a) bccryI
   (b) dbcryII
   (c) cryIAc
   (d) cryIdb
4. The first recombinant product to reach the market was:
   (a) Growth hormone
   (b) Tissue plasminogen activator
   (c) Factor VIII
   (d) Insulin
5. Biotechnology deals with:
   (a) Genetically modifying organism
   (b) Production of therapeutics
   (c) Production of better diagnosis
   (d) All of the above
6. Green revolution is:
   (a) Increase in yield of crops
   (b) Improved crop varieties
   (c) Lesser fertilizers and agrochemicals
   (d) All of these
7. Insecticidal protein cry does not kill bacillus because:
   (a) It is resistant to it.
   (b) The toxin is enclosed in vesicle.
   (c) The toxin is present in inactive form.
   (d) None of these.
8. DNA defects may be solved by:
   (a) Gene therapy
   (b) Replacement protein therapy
   (c) Stem cell therapy
   (d) All of these
9. The use of insect resistant crop would be:
   (a) The productivity would improve.
   (b) The usage of chemical agent would be reduced.
   (c) The environment and crop would be insecticide free.
   (d) All of the above.
10. Bioremediation can be helpful in:
    (a) Detoxifying waste material
    (b) Burying waste material
    (c) Burning waste material
    (d) None of these
11. Which of the following statements are true?
    (1) In all the cells of our body, all the genes are active.
    (2) In different cells of our body, different genes are active.
    (3) Gene expression is spatially and temporally regulated.
       (a) All 1, 2, and 3 are correct.
       (b) 1 and 2 are correct.
       (c) 1 and 3 are correct.
       (d) 2 and 3 are correct.
12. In a classic experiment, Dr. Edward Jenner demonstrated that:
    (a) Inoculation with monoclonal antibody was able to prevent small pox.
    (b) Inoculation with pus from sores due to cowpox could prevent small pox.
    (c) Attenuated vaccine was able to prevent small pox.
    (d) None of the above.

Answers

1. (c); 2. (a); 3. (c); 4. (d); 5. (d); 6. (d); 7. (c); 8. (a); 9. (d); 10. (a); 11. (d); 12. (b)

Review Questions

Q1. What are cry proteins? What is their importance?
Q2. Give some applications of biotechnology in agriculture.
Q3. What is your opinion about labeling of biotechnology-based food product as rDNA technology derived product?
Q4. What are applications of biotechnology in maintaining environment?
Q5. What is medical biotechnology?
Q6. What are the challenges faced by biotechnology industry?
Q7. What do you think about GM crops?

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Some Related Resources

http://ificinfo.health.org/backgrnd/BKGR14.htm
http://www.bio.org/aboutbio/guide1.html
http://www.bio.org/aboutbio/guide2000/guide00_toc.html
http://www.bio.org/aboutbio/guide3.html
http://www.bio.org/aboutbio/guide4.html
http://www.dec.ny.gov/energy/44157.html
http://www.ers.usda.gov/whatsnew/issues/biotech/define.htm
http://www.nal.usda.gov/bic/bio21
http://www.nature.com/nbt/press_release/nbt1199.html
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