Modern biotechnology has integrated several disciplines, varying from physics, chemistry, mathematics, biology, engineering, economics, law and management. The extraordinary developments occurring in biotechnology throughout the world has lead to significant changes in world commerce, as well as in human healthcare and agriculture sectors. Production of vaccines employing recombinant DNA methods is one example of its potential application. Pharmacologically active compounds from plants and marine organisms can now be isolated effectively using biotechnology. Progress in agricultural biotechnology will make our society less dependent on sustained harvest, which is often subjected to the vagaries of weather or climate.

This chapter makes you familiar with the developments in biotechnology field and its applications in health care and agriculture.
The development of technologies that use living organisms or substances derived from them for the benefit of mankind led to the establishment of field of biotechnology. Thus, biotechnology is now defined as “Any technological application that uses biological systems, living organisms or derivatives thereof to make or modify products or processes for specific uses” (Food and Agriculture Organization). Humans have for centuries practiced biotechnology. They have used biotechnology to make bread, cheese, and beers and wines, to breed strong and productive animals, and to increase the yield of crops by selecting seeds from particularly desirable plants. The modern biotechnology is based on the fundamental knowledge of biosciences and thus, truly interdisciplinary in nature (Fig. 1). It has already made its presence felt in our daily life in many ways and now providing new opportunities for career advancement.

1.1 Historical Perspectives

The making of curd, bread, wine, and beer originated in prehistoric kitchens and the process leading to the development of these products came to be known as fermentation. Around 2000 BC, the Egyptians knew that when crushed dates were stored, a pleasantly intoxicating material was produced at first but if the mixture was allowed to stand for a longer time, it turned sour to yield vinegar, the strongest acid known to antiquity. By 1500 BC, the use of germinated cereals (malt) for the preparation of beer from bread leaven (a mass of yeast) on a cereal dough and the formation of wine from crushed grapes, were established as technical arts in Mesopotamia, Palestine and Egypt. The ancients also observed that the formation of beer, wine or vinegar was followed by changes that led to the liberation of noxious odors, thus showing putrefaction for plant materials as compared to the more rapid decay of animal or human tissues. The practical arts of preserving animal foods – drying, smoking, curing, pickling in brine, and treatment with granular salt -- were well developed in the prehistoric Near East and Europe. The well-known mummification procedures in Egypt used the technique of dehydration with a mixture of salts, largely sodium carbonate.

By 18th century it was observed that fermentation could be classified into three groups on the basis of final products:

1. Evolution of gas
2. Formation of alcohol, and
3. Production of acid

Antoine Lavoisier provided the chemical basis for the nature of alcoholic fermentation by using analytical techniques for the quantitative estimation of carbon.
1.1.1 Microorganisms as causative agents of fermentation

In the early 19th century, Nicolas Appert, a French manufacturer of confectionery who distilled spirits and food products, described methods for preserving foods by putting them into tightly closed vessels that were then heated in boiling water – this marked the beginning of the canning industry. Gay Lussac examined Appert’s closed heated vessels and found that they lacked oxygen. This led to the belief that oxygen was necessary for fermentation. The construction of achromatic compound microscope demonstrated in 1837 that the agents of fermentation are living organisms. Charles Cagniard–Latour in 1838 described the involvement of brewer’s yeast in alcoholic fermentation based on: (i) its constant occurrence in fermentation, (ii) cessation of fermentation under the conditions that killed yeast, such as boiling, treatment with arsenate, etc. and (iii) it was evoked and increased by the process itself - a phenomenon that applies to living organisms only.

In 1857, Louis Pasteur published his first report on formation of lactic acid from sugar through fermentation. It was already known that lactic acid is produced from sugar and that addition of chalk to fermentation mixture markedly increases the amount of lactic acid produced. Using a microscope, Pasteur showed the presence of lactic yeast, which is the agent for the making of curd and confirmed the presence of L-lactic acid using a polarimeter. In 1860, he presented a detailed report on alcoholic fermentation and concluded:
(i) The act of fermentation is a phenomenon, which is unique, and very complex, as it can be a phenomenon correlated with life, giving rise to multiple products such as succinic acid, glycerin etc. all of which are necessary.

(ii) There is never any alcoholic fermentation without there being simultaneously the organization, development, and multiplication of the globules which are already formed.

(iii) He held the same views on lactic fermentation, butyric fermentation, fermentation of tartaric acid etc.

(iv) He did not comment on the chemical act of cleavage of the sugar.

(v) Fermentation appears to be a physiological phenomenon.

(vi) Fermentation is a consequence of anaerobic life.

Our ancestors have developed their own kitchen technologies with the help of fermenting bacteria, like Lactobacillus, Leuconostoc, Lactococcus, Enterococcus, Pediococcus etc leading to the development of a wide variety of yummy dishes that we relish today (Table 1). The preparation of curd is a household recipe known to us for centuries. You too can prepare curd using the following mother’s recipe (Fig. 2):

**Making of the curd**

Have you observed your mother making curd at home? Depending on the size of the family and average consumption, she has arrived at her own quantitative approach. Assuming a family of four consumes 200 ml/person of curd, she takes 800-1000 ml of milk, warms/cool it to lukewarm temperature (~37°C). Then she takes a teaspoon of the previous day’s curd and distributes it uniformly in one liter of milk by stirring it. Assuming that it is a warm summer day, the curd is ready in about four hours.

**Observations**

- The raw material, milk, has been converted completely into a semisolid product in four hours by addition of only one teaspoon of curd.
- The raw material, a white homogeneous slightly sweet liquid, has been converted into a semisolid, sour tasting product.
- There appears to be both a physical as well as a chemical change during the process.

The 19th century saw the growth of industries linked to fermentation, which gave rise to products like wine, beer, and whisky. This period also saw the growth of canned food industry, which helped in the preservation of food for a longer time and made them available during off-seasons. The fermentation industry along with agricultural practices of animal husbandry and plant hybridization led to the shifting of attention from agriculture to industry.

The 20th century saw man develop the ability to conquer several diseases thanks especially to the antibiotic revolution in the 1940s. The antibiotic revolution demanded large-scale production to meet the challenging demand of the health sector. This resulted in a shift from kitchen technologies to large-scale manufacturing in an industrial sector. And this further led to close interaction between fermentation
scientists and engineers from various disciplines of engineering, which gave rise to branches like bioprocess engineering, food science engineering and technology, bio-expert system engineering and artificial neural networks, validation engineering, metabolic engineering and environment management.

Table 1. Traditional lactic-fermented foods of India

<table>
<thead>
<tr>
<th>Food</th>
<th>Substrate</th>
<th>Nature and use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Traditional fermented products of south India</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idli</td>
<td>Rice-black gram</td>
<td>Steamed, spongy cake; breakfast food</td>
</tr>
<tr>
<td>Dosa</td>
<td>Rice-black gram</td>
<td>Spongy pan cake, shallow-fried; staple food</td>
</tr>
<tr>
<td>Ambali</td>
<td>Millet, rice</td>
<td>Steamed cake; staple food</td>
</tr>
<tr>
<td><strong>Traditional fermented products of north India</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bhallae</td>
<td>Black gram</td>
<td>Deep-fried patties; snack</td>
</tr>
<tr>
<td>Vadai</td>
<td>Black gram</td>
<td>Deep-fried; snack</td>
</tr>
<tr>
<td>Papad</td>
<td>Black gram</td>
<td>Circular wafers; snack</td>
</tr>
<tr>
<td>Wari</td>
<td>Black gram</td>
<td>Spongy cake; snack</td>
</tr>
<tr>
<td>Bhatura</td>
<td>Wheat</td>
<td>Flat deep-fried, leavened bread; snack</td>
</tr>
<tr>
<td>Nan</td>
<td>Wheat</td>
<td>Leavened flat backed bread; staple food</td>
</tr>
</tbody>
</table>

Fig. 2. Making of the curd
Jalebi | Wheat | Crispy, deep fried pretzel; sweet confectionery
Paneer | Milk | Soft milk-flavored cheese; fried curry
Dahi | Milk | Thick-gel, savory

**Traditional fermented products of western regions of India**

Dhokla | Bengal gram | Spongy cake; snack
Khaman | Bengal gram | Spongy cake; breakfast food
Rabadi | Wheat/Pear-millet /Barley-Buttermilk mixture | Cooked paste; staple food
Srikhand | Milk | Concentrated sweetened, savory

**Indigenous fermented products of eastern regions of India**

Mishti dahi | Milk | Thick gel; sweet savory
Tari | Date palm | Sweet cloudy white alcoholic beverage

**Traditional fermented products of the Himalayas**

Gundruk | Leafy vegetable | Sun-dried, sour-acidic taste; soup/pickles
Sinki | Radish tap root | Sun-dried, sour-acidic taste; soup/pickles
Mesu | Bamboo shot | Sour-acidic, pickles
Khalpi | Cucumber | Sour, pickles
Chhurpi | Milk | Soft mass, cheese-like, mild sour; curry

1.2. **Technology and applications of Biotechnology**

The current excitement about modern biotechnology is due to the manner in which this science has pushed organisms beyond their natural abilities by genetic engineering and hybridoma technology. Nature has equipped every organism with the capacity to perform within an optimum or balanced system. Some of the common Living systems with industrial applications are listed in Table 2. Modern biotechnology has tinkered with the genetic material of the organism by introducing foreign genes. The purpose is to push the organism to do things it never did before. Ability to genetically manipulate organisms right from viruses to mammals led to the genomics revolution – touted as the third technological revolution following the industrial and computer revolutions. For example, study of genomics has helped not only in determining the entire gene sequence but also making fine-scale genetic maps of organisms. Bioremediation technologies are used to clean our environment by removing toxic substances from contaminated soils and ground water. Agricultural biotechnology has reduced our dependence on pesticides. Manufacturing processes based on biotechnology has made it possible to produce paper and chemicals with less energy, less pollution, and less waste. Biotechnology has now found applications in virtually every sphere of human activity (Table 3). Different technologies used in biotechnology and their applications are as follows

**Table 2. Living systems with Industrial applications**

**Prokaryote**

**Eubacteria**

**Unicellular**
1.2.1 Bioprocessing Technology

Bioprocess technology is the oldest in all the biotechnologies. Bioprocessing technology, uses living cells or the molecular components to manufacture desired products. The living cells most commonly used are one-celled microorganisms, such as yeast and bacteria; the biomolecular components mostly used are enzymes, which are proteins that catalyze biochemical reactions. Microbial fermentation, is a form of bioprocess technology has been used for thousands of years—unwittingly—to brew beer, make wine, leaven bread and pickle foods. Now a day recombinant DNA technology coupled with microbial fermentation are used to manufacture a wide range of biobased products including human insulin, the hepatitis B vaccine, the calf enzyme used in cheese making, biodegradable plastics, and laundry detergent enzymes.

1.2.2 Cell Culture

Cell culture technology is the growing of cells outside of living organisms. Cell culture are of many types.

Plant Cell Culture: An essential step in creating transgenic crops, plant cell culture also provides us with an environmentally sound and economically feasible option for obtaining naturally occurring products with therapeutic value. Plant cell culture is also an important source of compounds used as flavors, colors and aromas by the food-processing industry.

Mammalian Cell Culture: Livestock breeding has used mammalian cell culture as an essential tool for decades. Eggs and sperm, taken from genetically superior bulls and cows, are united in the lab, and the resulting embryos are grown in culture before being implanted in surrogate cows.

1.2.3 Recombinant DNA Technology

Recombinant DNA technology is viewed as the cornerstone of biotechnology. The term recombinant DNA means the joining or recombining of two pieces of DNA from two different sources. Genetic modification using recombinant DNA techniques allows us to move genes whose functions are known. By making manipulations more precise and outcomes more certain, the risk of producing organisms with unexpected traits are decreased. Recombinant DNA technologies have various uses like research application, creation of genetically modified variety of plants and animals.
1.2.4 Cloning
Cloning technology allows us to generate a population of genetically identical molecules, cells, plants or animals. Because cloning technology can be used to produce molecules, cells, plants and some animals, its applications are extraordinarily broad. Molecular or gene cloning is the process of creating genetically identical DNA molecules. Molecular cloning is foundation of the molecular biology and is a fundamental tool of biotechnology research, development and commercialization. All applications in biotechnology, from drug discovery and development to the production of transgenic crops, depend on gene cloning.

Animal cloning has helped us to rapidly incorporate improvements into livestock herds for more than two decades and has been an important tool for scientific researchers. One of the technique used in animal cloning is Somatic cell nuclear transfer (SCNT). It involves transfer of the nucleus of a somatic cell taken from an adult female and transferred it to an egg cell from which the nucleus had been removed. The resulting cell behaved like a freshly fertilized zygote, which develops into an embryo.

1.2.5 Protein Engineering
Protein engineering technology involves improvement of existing proteins, such as enzymes, antibodies and cell receptors, and to create proteins not found in nature. This technique is used in conjunction with recombinant DNA techniques. Such engineered proteins are used in drug development, food processing and industrial manufacturing.

1.2.6 Biosensors
Biosensor technology couples the knowledge of biology with microelectronics. A biosensor is composed of a biological component, such as a cell, enzyme or antibody, linked to a tiny transducer—a device powered by one system that then supplies power (usually in another form) to a second system. Biosensors are detecting devices that rely on the specificity of cells and molecules to identify and measure substances at extremely low concentrations. When the substance of interest binds with the biological component, the transducer produces an electrical or optical signal proportional to the concentration of the substance. Biosensors can be used for; measurement of nutritional value, freshness and safety of food; location and measurement of environmental pollutants.

1.2.7 Nanobiotechnology
The word nanotechnology derives from nanometer, which is one-thousandth of a micrometer (micron), or the approximate size of a single molecule. Nanotechnology is the study, which involves manipulation and manufacture of ultra-small structures and machines made of as few as one molecule. Nanobiotechnology uses the knowledge of nanotechnology and biomolecules of cell to produce desired technology. Some applications of nanobiotechnology includes; fast diagnosis of disease, bio-nanostructures creation for getting functional molecules into cells, improvement of specificity and timing of drug delivery.

<table>
<thead>
<tr>
<th>Health Industry</th>
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</thead>
<tbody>
<tr>
<td>• Protein pharmaceuticals</td>
</tr>
</tbody>
</table>
• Vaccine and Therapeutic agents
• Diagnostics (Protein- or DNA)
• Gene Therapy

**Agriculture Industry**
• Biopesticides/ Biofertilizers
• Crops tolerant to abiotic and biotic stresses-
• Pharmaceutical production

**Chemical Industry**
• Fermentation process to produce organic chemicals
• Production of high purity chemicals
• Use of energy efficient processes

**Cleaning Industry**
• Use of enzymes as detergents like proteases

**Textile Industry**
• Use of enzymes for finishing of fabrics
• Use of genetically-modified cotton

**Pulp and Paper Industry**
• Improvement of physical properties of fibers\n• Biobleaching of pulp

**The advent of the 21st century has witnessed the:**
• Mapping of genomes of many plant and animal species including humans,
• Cloning of animals including many higher mammals,
• Development of target-specific new molecules and drugs,
• Stem cell therapy for genetic diseases and accident cases,
• Tissue engineering for producing artificial tissues and organs,
• Creation of transgenic animals and plants for the production of recombinant biopharmaceuticals,
• Emergence of bioinformatics and computational biology in understanding the functioning of cells and organs at genomic and protein levels etc.,
• Application of in-silico experiments to understand pharmacology, toxicity etc.,
• Development of automated gene and protein sequencing
• Identification of proteins and peptides by mass spectrometry,
• Evolution of nanotechnology and its application in nanomedicine, and
• Growth of RNA interference biology

1.3 **Global Market of Biotech Products**

The biotechnology industry is also improving lives through its substantial economic impact. Biotechnology has stimulated the creation and growth of small business, generated new jobs, and encouraged agricultural and industrial innovation. In USA, the industry currently employs
over 150,000 people and invests ~ $10 billion a year on research and development (Table 4).

Table 4. U.S. biotechnology product sales forecast (Value in $ million)

<table>
<thead>
<tr>
<th>Key sectors</th>
<th>Base year 1998</th>
<th>Forecast year 2003</th>
<th>Forecast year 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human therapeutics</td>
<td>9,120</td>
<td>16,100</td>
<td>27,000</td>
</tr>
<tr>
<td>Human diagnostics</td>
<td>2,100</td>
<td>3,100</td>
<td>4,300</td>
</tr>
<tr>
<td>Agriculture</td>
<td>420</td>
<td>1,000</td>
<td>2,300</td>
</tr>
<tr>
<td>Specialties</td>
<td>390</td>
<td>900</td>
<td>2,000</td>
</tr>
<tr>
<td>Non-medical diagnostics</td>
<td>270</td>
<td>400</td>
<td>600</td>
</tr>
</tbody>
</table>

Modern biotechnology products started coming into the market only in the late 1980s. Globally, the share of biotechnology in the pharmaceutical market was less than 0.14% in 1985. By 2000 this share grew to 20%. Today, about 60% of the biotechnology products in the market are healthcare products (Table 5). About 21% of the biotechnology products go into agriculture and animal husbandry. The commonly sought after agronomic traits for plants are given in Table 6. Given that there are some crucial questions that have remained unanswered so far (for example, how a bacterially produced human protein would acquire the same three-dimensional structure as found in the human system?), remain a challenge and enigma for biotechnologists.

Table 6. Agronomic traits from Biotechnology

- Herbicide tolerance
- Insect resistance
- Disease resistance
- Abiotic stress, e.g., Water, Temperature, Salt, Metals
- Value added traits, e.g., Oil, Vitamins, Minerals
- Nutritional quality, e.g., proteins, fats, carbohydrate; Plant properties, e.g., shelf-life, flavors
fragrance

• Allergen reduction
• Nitrogen fixation
• Yield
• Pharmaceutical production, e.g., Vaccines, Proteins

2001: GMO 130 MM acres (75% U.S.),
  e.g., cotton, soybean, corn, canola, rice

Overall Benefits: Less cost / acre, More yield, Less pollution

1.4 Public Perception of Biotechnology

The public demands that biotechnology should work for the benefit of the society. This entails major changes in the sciences and the social sciences. But these changes are not possible without active societal participation. The participation of the society has become all the more important because of the realization of the environmental consequences of science and technology. A technology is democratic if it has been designed and chosen with democratic participation. Participation in scientific research decision-making and policy-making, in general, requires certain discretion and deliberative capabilities on the part of the citizens. Civil society organizations (CSOs) have inherent advantages in handling information (on social, economic and ecological variables and processes) and controlling decision processes. The debate about the relative merits of representative participation and direct democratic participation of the individual in environmental decisions remains unresolved. It is argued that representative participation may be the solution for the time being, because detailed debates on technology or the ‘technology tribunals’ work only in contexts where there are literate and informed public and a liberal democratic government. Therefore, the participation should begin at all levels of decision-making so that the society could have better and more informed citizens.

While developments in science and technology are instrumental in most of our progress, some genuine concerns have been raised regarding the long-term consequences on our health and environment. In 1985, the UN assembly created the Brundtland Commission also called WCED (World Commission on Environment and Development). The commission submitted a report on the theme of sustainable development. It recognized that there is a real conflict between meeting the needs and desires of 6 billion people living today and the possibility of satisfying the ten billion people expected to inhabit the Earth by 2050!! The WCED defined Sustainable Development as follows:

“Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs.”

Definitions of sustainable development are abundant and are interpreted in varying manner by different nations. In all these definitions and the way they have been implemented, science and technology is invariably portrayed as a double-edged sword. A heated debate is taking place globally over the issue of GM crops. This debate, which features science, economics, politics and even religion, is taking place almost everywhere. Similar is the case with embryonic stem cell research and its funding.
1.5 Biotechnology in India and Global Trends

1.5.1 Indian scenario
The Indian response to the biotechnology industry and its products has been very supportive right from the early 1980s when the investments in the Department of Biotechnology (DBT) (http://dbtindia.nic.in) and other research and development avenues were actively promoted. The Government has set up a Technology Development Board to promote product development with universities, industries and national institutes. Vision 2020 document has been prepared by Technology Information, Forecasting and Assessment Council (TIFAC), which also includes biotechnology. Indigenously developed Hepatitis B vaccine has been developed, manufactured and marketed since 1998. There are nearly 30 companies devoted to development of modern biotechnology products in India. Currently, India’s share in the global biopharmaceutical market is just 1.5% and is valued at US $ 1.05 billion. By registering a high growth rate of ~32 per cent in recent years, India is poised to become a major player in the coming years.

1.5.2 Global scenario
The present global biopharmaceutical industry is valued at US $ 71 billion. With the present growth rate of 16 per cent, it is likely to cross $ 100 billion mark by the year 2010. USA promotes biotechnology enterprise by gearing up the administration to develop policies, which will foster biotechnology innovations as expeditiously and prudently as possible. There has been steady increase in support for basic scientific research at the National Institutes of Health (NIH) and other science agencies. The process of approving new medicines, making them available and encouraging private-sector research investment and small business development are being accelerated through new tax incentives and small business innovations research program. The Government promotes intellectual property protection and open international markets for biotechnology inventions and products; and developed public databases that enable scientists to coordinate their efforts in an enterprise that has become one of the world’s finest examples of partnership among university-based researchers, government and private industry. In addition, the administration has strengthened efforts to improve science education and promote the freedom of scientific enquiries. It has also provided guidelines to protect patients from the misuse or abuse of sensitive medical information and provide Federal regulatory agencies with sufficient resources to maintain sound, science-based review and regulation of biotechnology products. It has also ensured that science-based regulatory programs worldwide promote public safety, earn public confidence and guarantee fair and open international markets. The European and the US biotechnology industries both have around 2000 companies, but the US sector employs nearly twice as many people, spends around three times as much on research and development, has twice the number of employees involved in research and development. It also earns twice as much revenue compared to the European counterpart.

Review Questions
1. Do you agree that your grandmother unknowingly practiced a technique of biotechnology? Describe the technique.
2. What are the two instruments Louis Pasteur used to show the presence of lactic yeast and L-lactic acid?
3. Name four traditional fermented foods of India.

4. Name some of the living systems that are being used for the industrial applications.

5. List the important applications of biotechnology in health care.

6. List the important applications of biotechnology in agriculture.

7. Which was the first recombinant DNA-based product produced and marketed in India?

Further Reading


