# MODERN CONCEPTS of BIOTECHNOLOGY

HD KUMAR

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# MODERN CONCEPTS OF BIOTECHNOLOGY

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## **Preface**

Many universities have introduced Biotechnology in their curricula and those that have not are contemplating to do so in the near future. Although several books on biotechnology have been published in the past two decades, most are either multi-author edited volumes or deal with one or a few areas in depth rather than covering a broad spectrum of topics in a general introductory manner. Furthermore, many of these books are prohibitively expensive and beyond the reach of students in developing countries.

Having been engaged in the teaching of a Biotechnology (especially microbial) course and in research on applied phycology and microalgal biotechnology for the last several years, I have written this book to cater to the needs of undergraduate students and readers from a diverse background, with special reference to those in developing countries which are usually very rich in biological diversity but quite poor in biotechnology. It is hoped that this book may go a small way in correcting at least some of this imbalance and mismatch between biodiversity and biotechnology in the underdeveloped world.

Virtually all aspects of biotechnology and allied areas are covered concisely. I have attempted to bring out linkages and interfaces between biotechnology on the one hand, and general science, sociology, ecology, economics, environment, agriculture, industry, medicine, energy, poverty, pollution, business, marketing, biosafety, intellectual property rights and patenting issues, on the other hand. In this attempt, some overlap has crept in here and there, but the overall objective has been to make the book particularly useful for students in many countries.

I wish to thank the following for their direct or indirect support and help in the production of this book: M Ohmori (Tokyo), DL Parker (Oshkosh, Wisconsin), DP Häder (Erlangen, Germany), S. Varadarajan (Delhi), Ashok Kumar, LC Rai and JB Singh (Varanasi), Council of Scientific and Industrial Research, Department of Science and Technology, and Indian National Science Academy (New Delhi).

October, 1997

**HD Kumar** 

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# **GENERAL INTRODUCTION**

### **DEFINITION AND HISTORY**

Biotechnology has been defined in several different ways during the last ten years. The differences in the definition stem from the fact that biotechnology is a multi-disciplinary applied science. There is considerable agreement that certain processes, which utilize living systems, definitely are biotechnology; what is less certain is which processes are not biotechnology.

The term biotechnology is the short from of "biological technology". It is used to describe both biochemical engineering and biomedical engineering.

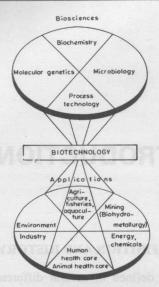
One of the most popular definitions of biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services. "In this, the word "agent" denotes a wide range of biological things such as enzymes, whole cells or multi-cellular organisms. Services and goods mean such processes as waste and water treatment. The scientific and engineering principles are chiefly microbiology, biochemistry, genetics, and biochemical and chemical engineering.

Another definition of biotechnology is the use of biological organisms or their constituents for the transformation of inputs into commercial outputs.

Biotechnology is a multidisciplinary field involving biochemistry, molecular biology, genetics, immunology, microbiology, pharmacology, fermentation and agriculture, to name just a few. Each of the contributing subject areas brings its own special vocabulary and nomenclature standards and considerable difficulties of communication can sometime result.

Fig 1-1 illustrates the multidisciplinary perspective of biotechnology and its contacts and applications.

Customarily, conventional agriculture or animal breeding are not included within biotechnology but inevitably there is some overlap with agriculture, both as a supplier of raw materials and in genetic improvement of crop plants. In a broad sense, biotechnology may be understood as referring to the commercialization of biological processes. i.e., scaling up biological process from a test tube or small flask to the level of a fermenter or wastewater treatment plant. Biotechnology is thus not an industry defined by products or services such as textiles or automobiles, It refers to the use of microbial, animal or plant cells and enzymes to synthesize, degrade, or transform materials. Its



**Fig. 1.1** Contacts of biotechnology with some disciplines of biosciences, and the applications of biotechnology.

products and services range from foods to fuels, from waste disposal to pharmaceuticals. In other words, you may define modern biotechnology as the application of recently developed skills in microbial and biochemical technology to applied biology, i.e., to the exploitation of biological systems and processes for our own use. One of the most commonly used techniques in biotechnology is genetic engineering. By using this technique we can alter or manipulate cells to perform certain functions more beneficially, that is to produce more or better products or to do so with greater efficiency.

Biotechnology is by no means a new discipline. It has been with us since times immemorial. The roots of modern biotechnology lie in the fermentations of foods and drinks. These industries span almost every society and have evolved over centuries. The selection and improvement of microbial strains for the efficient large-scale production of antibiotics has been carried out for some 50 years now. Large-scale fermentations by fungi and bacteria have been in use for many decades for the production of a wide variety of products. One of the earliest examples of the older (traditional) biotechnology is the industrial manufacture of beer. The modern biotechnology is in many ways similar to the older biotechnology; we have merely developed advanced and better ways for the selective manipulation of the genetic material of not only useful microorganisms but also of the cells of higher plants and animals. The core of most modern biotechnological processes is the conversion of relatively cheap raw material into highly valuable products or services.

Biotechnology is now a mature but explosive technology which has been reborn following the elucidation of the structure of nucleic acids that led to the development of advanced techniques for selectively changing the genetic composition of cells through genetic engineering, development of monoclonal antibodies and hybridomas, and somaclonal variation of plant cells.

Biotechnology has a major impact on basic human needs engendered in the market segments of : health care, agriculture, forestry; food ingredients, industrial chemicals, plastics; energy, mining, pollution control, and bioelectronics. Innovations in health care aimed at very high-value therapeutic proteins are currently in the forefront and crop agriculture comes a far second. In agricultural biotechnology intensive research in

molecular and cell biology has led to development of new gene transfer methods specially designed to overcome limitations of the early, traditional plant breeding, tools. This has brought within reach the capability to engineer most plant species. Innovations in pollution control are emerging strongly as a result of increased public and regulatory pressure.

The cluster of techniques that constitute new or modern biotechnology include genetic engineering, bioprocessing, monoclonal antibodies; protein engineering, bioinformatics, tissue culture; biological sensors, protoplast fusion, cell catalysis; biocatalytic reactors, and computer linkage of reactors and processes.

During the last two decades, it was widely expected that the new biotechnology would open up a new world of opportunities and increased profits. While biotechnology contributed to new processes and products in diverse economic sectors, the pace and extent of innovation during the last decade has disappointed many.

While biotechnology has generated commercial interest and investment, a number of constraints are influencing the pace and direction of innovation. Both large and small firms are affected, but new biotechnology firms find it much more difficult than older established firms to withstand financial pressures and get final products to market. Biotechnology is certainly not another electronics industry; it has to contend with major technical, regulatory, patent and marketing risks.

In the area of agriculture and food-related business, a number of specific areas were identified during the 1980s as being potential targets for biotechnology applications. Process improvements were expected in plant breeding, and it was thought that genetic engineering would result in new transgenic crops with insect and disease resistance. In the area of crop protection, new biological control agents for herbicide resistance would help prevent agrochemical companies decline into low value commodity producers. In animal breeding and health, improved disease control, embryo transfer and productivity enhancers promised increased efficiency.

Figure 1.2 illustrates the general features of any biotechnological process.

Even today the output of food and drink industries in volume and value greatly exceeds the modern fermentation processes for the production of drugs, amino acids, chemicals and industrial ethanol.

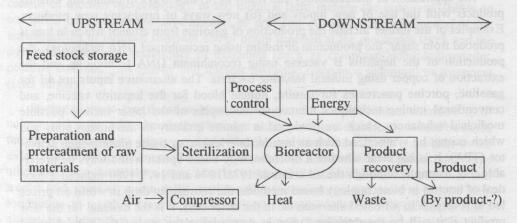


Fig. 1.2 A flow chart of any biotechnology process with emphasis on the different phases of operation.

The following three events during the last few decades exerted a profound influence on the development of modern biotechnology:

- 1. The sharp increase in the prices of crude oil and fossil fuels (such as petroleum, gasoline, etc).
- The use of restriction endonucleases and ligases brought within our reach the ability to cut DNA and rejoin it in new combinations; i.e., to manipulate the genetic material artificially.
- 3. The demonstration in 1975 by Köhler and Milstein of the production of monoclonal antibodies from the fusion of lymphocytes and myeloma and tumour cells.

The consequences and impact of these three developments are described in the next section. Fig. 1.3 shows several current technologies being applied in various biotechnology processes.

DNA probes find application in "genetic fingerprinting" as they aid the identification of individuals from samples of their cells, blood, skin or semen. These probes can be used in forensic medicine and to resolve paternity and immigration disputes.

During the last decade, strong linkages have been created between advances in biotechnology and those in microelectronics. New information processing technologies have produced a major impact on the efficiency of biotechnological processes. Examples are found in the use of microprocessors and computers in automated control of bioreactors and DNA synthesizers. Conversely, biotechnology has begun to exert some effect, albeit small, on information processing. For example, one area of application of protein engineering is in the field of biosensors and biochips where integrated circuit technology is combined with protein engineering technology.

### **NEW PRODUCTS AND TECHNIQUES**

The application of biotechnology can result in (a) new ways of producing existing products with the use of new inputs and (b) new ways of producing new products. Examples of the former include the production of gasoline from ethanol which in turn is produced from sugar; the production of insulin using recombinant DNA technology; the production of the hepatitis B vaccine using recombinant DNA technology and the extraction of copper using mineral leaching bacteria. The alternative inputs are oil for gasoline, porcine pancreases for insulin, human blood for the hepatitis vaccine, and conventional mining techniques for copper. Examples of the latter include possible medicinal substances which are produced in minute quantity in the human body and which cannot be synthesized such as insulin, interleukin, or tissue plasminogen activator (TPA). It is not clear whether a biotechnology based option will always be preferable. For example, although the oil crises of 1973/1974 and 1979/1980 induced a good deal of interest in biotechnology based methods, the subsequent drop in world oil prices led to reversion to oil-based alternatives. In the latter case, it is the demand for the new product that will be the decisive factor in determining the use of the biotech-based inputs (Fransmann, 1989).

The advent and applications of recombinant DNA and hybridoma technologies, plant and animal tissue culture, the metabolic abilities of microorganisms and the new

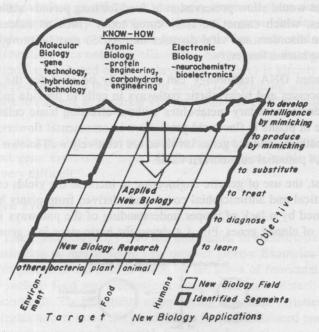


Fig. 1.3 Some current technologies used in biotechnology.

enzyme technologies have already revolutionized the chemical industry in the developed countries by providing cheaper, better and less polluting ways of manufacturing useful chemical substances. About 1200 firms in 19 countries (but none in India) are engaged in the development of the technologies for, and/or production of nearly a hundred important proteins through engineering.

In vitro fertilization has made it possible to establish pregnancies in thousands of women. Embryo transfer technology can revolutionize animal husbandry. Some applications of modern biotechnology in the medical field include the enhanced ability to transplant organs and the development of artificial organs as a consequence of the understanding of immune response and of organ function at the molecular level, and the development of new techniques of diagnosis (such as computer aided tomography, positron emission tomography and nuclear magnetic resonance scanning) or treatment (laser surgery).

During the next few decades, biotechnology would have overtaken chemical technology, and many such chemicals which are today produced chemically would be made through biotechnology. Many products of daily use would be cheaper and better to the extent that it all might even change our life styles. Amino acids, vitamins and some other nutrients essential for man may be produced cheaply and the cheapest diet that would take care of one's daily nutritional requirements would be a synthetic one. Production of alcohol from cellulose through its enzymatic hydrolysis could make alcohol so cheap that alcohol-based industry would be revolutionized. Availability of biodegradable plastics and home diagnostic kits would make life quite different from what it is today, as would be the ability to determine the sex of early embryos (including human embryos), development of new species (specially plants), and availability of

techniques that would allow preservation of food for long periods without refrigeration. Some diseases, which cannot be fully cured today (such as cancer, leprosy, heart diseases, brain disorders and viral diseases like AIDS) may be brought under control, thus increasing human longevity.

Recombinant DNA technology can potentially be used for the manipulation of metabolic processes and biosynthetic pathways in cells to provide increased yields of desirable products/secondary metabolites without affecting basic cellular functions. A good example in plants is flavonoid production in ornamental flowers; in this case the biosynthetic pathway and the genes involved are relatively well known and any changes in colour are of potential commercial value.

In contrast, the use of genetic engineering to increase the yields of a large number of pharmaceutical and antimicrobial compounds derived from plants has until recently been constrained by a lack of proper understanding of the pathways involved and the unavailability of cloned genes. Fig. 1.4 shows the basic steps in a gene cloning experiment.

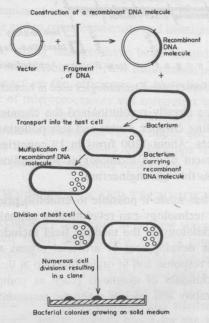


Fig. 1.4 The basic steps in gene cloning.

The technique called "biolistics" involves shooting DNA coated metal particles directly into living target cells. The introduced DNA is expressed within days after transfer and, rarely, becomes stably integrated into the plant genome. This approach has been used to test for transient expression of foreign genes in non-morphogenic tissues of dicots and monocots. This approach is being applied to embryogenic targets with a view to obtaining stably transformed fertile plants, in particular for the cereals.

The biolistic process is by no means a "wonder weapon" that can solve all the transformation problems with grain legumes, cereals and other difficult crops. It is

desirable to judge whether or not to use this new approach since many pretests and adaptations are necessary for every type of target of a given species. However, if a protoplast-to-plant system is not available for a particular plant, and *Agrobacterium*-mediated transformation does not work efficiently, and provided that an *in vitro* regeneration system (preferably based on an embryogenic culture) is already available, then the biolistic approach becomes the method of choice for obtaining stable transformants, particularly if one has access to a marker system mediating efficient selection (Mendel, 1990).

The biolistic process also has the potential to become a good tool in tests for tissuespecific transient gene expression, especially in plants where stable transformation is not possible or very difficult.

### **Extension to New Products**

In several cases, biotechnology has led companies in industrialized countries to extend their production or marketing to new product groups. Examples are the shift by agrochemical companies in the area of seeds, or the move of fermentation-based companies in areas such as food and alcoholic beverages into new biotech-based products such as pharmaceuticals. The possibility of producing new plant varieties that are herbicide-resistant creates new commercial possibilities for herbicide-seed packages. In order to increase returns from this new potential, large agrochemical companies have started acquiring or establishing seed companies. The possibility of developing pest-resistant plants has implications for the development of pesticides, enabling the merger of agrochemical and seed research, production and sales activities (OTA, 1984; Dembo and Morehouse, 1987).

### CURRENT DEVELOPMENTS AND FUTURE PRIORITIES

Some current developments in biotechnology are listed below. In the food and agriculture sector, there is promise of:

- 1. Crops with inherent protection from insect pests and resistance to viral and fungal disease.
  - 2. Better nutritional value of food crops.
  - 3. Crops with enhanced temperature, salinity or drought tolerance.
  - 4. Wider genetic diversity in plants and animals.
  - 5. Diagnostic agents for plant and animal disease, and
  - 6. Improved animal breeding techniques.

In the sphere of environment, improvement is expected in:

- 1. Resource recovery and recycling.
- 2. Waste water purification.
- 3. Hazardous waste management.
- 4. Biodegradable plastics and packaging, and
- 5. Reduced agricultural waste.

In the areas of human health and welfare, biotechnology can facilitate health promotion through:

- 1. Human growth hormones to treat nutritional deficiencies.
- 2. Human insulin to treat diabetes.
- 3. Tissue plasminogen activator (TPA) to treat blood clots and heart attacks.
- 4. Interferons to treat leukemia, viral diseases and cancer. and
- 5. Vaccines for hepatitis B and other diseases.

There are three chief priorities for biotechnology research:

- To discover, characterize, modify, and control the genetics and biochemical products and processes of a broad range of terrestrial and marine organisms for application in biotechnology.
  - 2. To apply the tools of modern biotechnology to problems in agriculture, the environment, and manufacturing to facilitate the development of new and improved products, processes, and test methods.
  - To strengthen and enhance facilities, repositories, data-bases, and human resources to ensure the future vitality of the biotechnology enterprise.

The following is a summary of the priorities identified in each area:

### A. Agricultural biotechnology

- Continue mapping and sequencing of animal/plant/microbial genomes to elucidate gene function and regulation and to facilitate the discovery of new genes as a prelude to gene modification.
- Determine biochemical and genetic control mechanisms of metabolic pathways in animals, plants, and microbes that may lead to products with novel food, pharmaceutical, and industrial uses.
- 3. Extend understanding of the biochemical and molecular basis of growth and development including structural biology of plants and animals.
- 4. Elucidate the molecular basis of interactions of plants and animals with their physical and biological environments, to improve the organisms' health.
- 5. Enhance food safety assurance methodologies, such as rapid tests for identifying chemical and biological contaminants in food and water.

### **B.** Environment

- 1. Studies of the structure of microbial communities and their dynamics in response to normal environmental variation and novel anthropogenic stresses.
- Determine the biochemical mechanisms, including enzymatic pathways, involved in anaerobic degradation of pollutants.
- 3. Strengthen research in microbial genetics for enhancing the capabilities of microorganisms to degrade pollutants.