1st Lecture

Long before we knew that microorganisms existed or that genes were the units of inheritance, humans looked to the natural world to develop methods to increase food production, preserve food, and heal the sick. They discovered that grains could be preserved through fermentation into beer; saved part of bread dough to the next day, and that intentional exposure to a "contagion" could somehow provide protection from an infectious disease on subsequent exposure. Since the discovery of the microscopic world in the 17th century, microorganisms have been employed in the development of numerous useful processes and products. Many of these are found in our households and backyards. Lactic acid bacteria are used to prepare yogurt and probiotics, insecticide-producing bacteria are sprayed on many of the plants from which the vegetables in our refrigerator were harvested, nitrogen- fixing bacteria are added to the soil used for cultivation of legumes, the enzymatic stain removers in laundry detergent came from a microorganism, and antibiotics derived from common soil microbes are used to treat infectious diseases. These are just a few examples of traditional biotechnologies that have improved our lives.

Up to the early 1970s, however, traditional biotechnology was not a well-recognized scientific discipline, and research in this area was centered in departments of chemical engineering and occasionally in specialized microbiology programs.

In a broad sense, biotechnology is concerned with the production of commercial products generated by the metabolic action of microorganisms.

More formally, biotechnology may be defined as "the application of scientific and engineering principles to the processing of material by biological agents to provide goods and services." The term "biotechnology" was first used in 1917 by a Hungarian engineer, Karl Ereky, to describe an integrated process for the large-scale production of pigs by using sugar beets as the source of food. According to Ereky, biotechnology was "all lines of work by which products are produced from raw materials with the aid of living things." This fairly precise definition was more or less ignored. For a number of years, the term biotechnology was used to describe two very different engineering disciplines. On one hand, it referred to industrial fermentation. On the other, it was used for the study of efficiency in the workplace what is now called ergonomics. This ambiguity ended in 1961 when the Swedish microbiologist Carl Göran Hedén recommended that the title of a scientific journal dedicated to publishing research in the fields of applied microbiology and industrial fermentation be changed from the Journal of Microbiological and Biochemical

Engineering and Technology to Biotechnology and Bioengineering. From that time on, biotechnology has clearly and irrevocably been associated with the study of "the industrial production of goods and services by processes using biological organisms, systems, and processes," and it has been firmly grounded in expertise in microbiology, biochemistry, and chemical engineering.

An industrial biotechnology process that uses microorganisms for producing a commercial product typically has three key stages:

1. Upstream processing: preparation of the microorganism and the raw materials required for the microorganism to grow and produce the desired product.

2. Fermentation and transformation: growth (fermentation) of the target microorganism in a large bioreactor (usually >100 liters) with the consequent production (biotransformation) of a desired compound, which can be, for example, an antibiotic, an amino acid, or a protein

3. Downstream processing: purification of the desired compound from either the cell medium or the cell mass Biotechnology research is dedicated to maximizing the overall efficiency of each of these steps and to finding microorganisms that make

products that are useful in the preparation of foods, food supplements, and drugs. During the 1960s and 1970s, this research focused on upstream processing, bioreactor design, and downstream processing. These studies led to enhanced bioinstrumentation for monitoring and controlling the fermentation process and to efficient large-scale growth facilities that increased the yields of various products.

The biotransformation component of the overall process was the most difficult phase to manipulate. Commodity production by naturally occurring microbial strains on a large scale was often considerably less than optimal. Initial efforts to enhance product yields focused on creating variants (mutants) by using chemical mutagens or ultraviolet radiation to induce changes in the genetic constitution of existing strains. However, the level of improvement that could be achieved in this way was usually limited biologically. If a mutated strain, for example, synthesized too much of a compound, other metabolic functions often were impaired, thereby causing the strain's growth during large-scale fermentation to be less than desired. Despite this constraint, the traditional "induced mutagenesis and selection" strategies of strain improvement were extremely successful for a number of processes, such as the production of antibiotics.

The traditional genetic improvement regimens were tedious, time consuming, and costly because of the large numbers of colonies that had to be selected, screened, and tested. Moreover, the best result that could be expected with this approach was the improvement of an existing inherited property of a strain rather than the expansion of its genetic capabilities.

Despite these limitations, by the late 1970s, effective processes for the mass production of a wide range of commercial products had been perfected.

Today, we have acquired sufficient knowledge of the biochemistry, genetics, and molecular biology of microorganisms to accelerate the development of useful and improved biological products and processes and to create new products that would not otherwise occur. Distinct from traditional biotechnology, the modern methods require knowledge of and manipulation of genes, the functional units of inheritance, and the discipline that is concerned with the manipulation of genes for the purpose of producing useful goods and services using living organisms is known as molecular biotechnology.

The pivotal development that enabled this technology was the establishment of techniques to isolate genes and to transfer them from one organism to another. This technology is known as recombinant deoxyribonucleic acid (DNA) technology, and it began as a lunchtime conversation between two scientists working in different fields who met at a scientific conference in 1973. In his laboratory at Stanford University in California, Stanley Cohen had been developing methods to transfer plasmids, small circular DNA molecules, into bacterial cells. Meanwhile, Herbert Boyer of the University of California at San Francisco was working with enzymes that cut DNA at specific nucleotide sequences. Over lunch at a scientific meeting, they reasoned that Boyer's enzyme could be used to splice a specific segment of DNA into a plasmid and then the recombinant plasmid could be introduced into a host bacterium using Cohen's method.

FIGURE 1.2 Many scientific disciplines contribute to molecular biotechnology, which generates a wide range of commercial products.

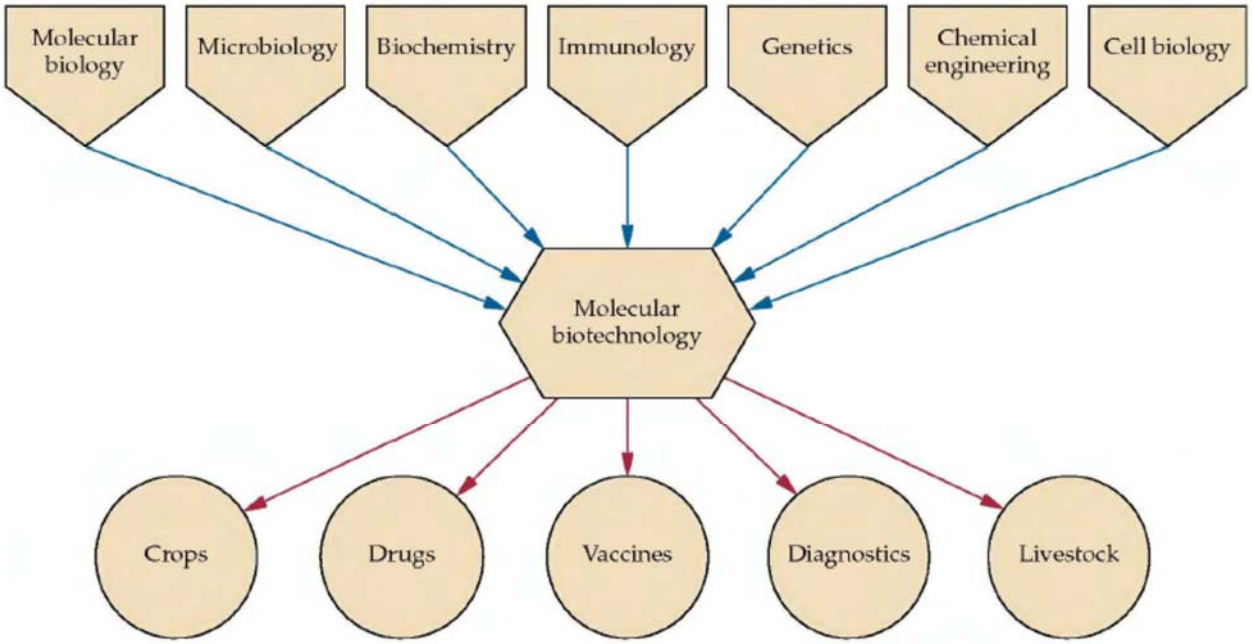

TABLE 1.1 Selected developments in the history of molecular biotechnology

Date	Event
1917	Karl Ereky coins the term "biotechnology"
1940	A. Jost coins the term "genetic engineering"
1943	Penicillin is produced on an industrial scale
1944	Avery, MacLeod, and McCarty demonstrate that DNA is the genetic material
1953	Watson and Crick determine the structure of DNA
1961	The journal *Biotechnology and Bioengineering* is established
1961–1966	Entire genetic code is deciphered
1970	First restriction endonuclease is isolated
1972	Khorana and coworkers synthesize an entire tRNA gene
1973	Boyer and Cohen establish recombinant DNA technology
1975	Kohler and Milstein describe the production of monoclonal antibodies
1976	First guidelines for the conduct of recombinant DNA research are issued
1976	Techniques are developed to determine the sequence of DNA
1978	Genentech produces human insulin in *E. coli*
1980	U.S. Supreme Court rules in the case of Diamond *v.* Chakrabarty that genetically manipulated microorganisms can be patented
1981	First commercial, automated DNA synthesizers are sold
1981	First monoclonal antibody-based diagnostic kit is approved for use in the United States
1982	First animal vaccine produced by recombinant DNA methodologies is approved for use in Europe
1983	Engineered Ti plasmids are used to transform plants
1988	U.S. patent is granted for a genetically engineered mouse susceptible to cancer
1988	PCR method is published
1990	Approval is granted in the United States for a trial of human somatic cell gene therapy
1990	Human Genome Project is officially initiated
1990	Recombinant chymosin is used for cheese making in the United States
1994–1995	Detailed genetic and physical maps of human chromosomes are published
1994	FDA announces that genetically engineered tomatoes are as safe as conventionally bred tomatoes
1995	First genome sequence of a cellular organism, the bacterium *Haemophilus influenzae*, is completed
1996	First recombinant protein, erythropoietin, exceeds $1 billion in annual sales
1996	Complete DNA sequence of all the chromosomes of a eukaryotic organism, the yeast *Saccharomyces cerevisiae*, is determined
1996	Commercial planting of genetically modified crops begins
1997	Nuclear cloning of a mammal (a sheep) with a differentiated cell nucleus is accomplished
1998	FDA approves first antisense drug
1999	FDA approves recombinant fusion protein (diphtheria toxin–interleukin-2) for cutaneous T-cell lymphoma
2000	*Arabidopsis* genome is sequenced
2000	Monoclonal antibodies exceed $2 billion in annual sales
2000	Development of "golden rice" (provitamin-A-producing rice) is announced
2000	Over $33 billion is invested in U.S. biotechnology companies
2001	Human genome is sequenced
2002	Complete human gene microarrays (gene chips) become commercially available
2002	FDA approves first nucleic acid test system to screen whole blood from donors for HIV and HCV
2004	Large-scale sequencing of the Sargasso Sea metagenome begins
2005	NCBI announces that there are 100 gigabases of nucleotides in the GenBank sequence database
2006	Recombinant cancer vaccine becomes available to protect against cervical cancer
2008	Two-billionth acre of genetically engineered crops is planted
2009	FDA approves first drug produced in a genetically engineered animal (a goat)