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BIOTECHNOLOGY: AN OVERVIEW OF TECHNIQUES, RESEARCH AND APPLICATIONS

Introduction

The term biotechnology encompasses an expanding repertoire of techniques that use living organisms or parts of organisms to make or modify products, improve plants or animals, or develop micro-organisms for specific uses. Some common examples include vaccine development and production of insulin, hormones, and drugs for cancer therapies. In this paper, we review some of the technologies used in biotechnology facilities. By necessity, our approach is illustrative; but we attempt to examine the broad base of biotechnology and identify the powerful methodologies which have propelled its growth from a few centers of research and limited production in the 1960s to thousands of research and commercial establishments worldwide today.

Any attempt to subdivide biotechnology into categories or areas of emphasis is, to some degree, arbitrary. We will present a description of the scope of biotechnology which reflects its historical development. We begin with the classical techniques of culturing and fermentation of microorganisms and the use of these processes today for production of medical and agricultural products. Next, we will discuss the use of more complex organisms and animals in disease research and vaccine production. Finally, we will review the science and technology of gene manipulation (often called "genetic engineering"), a flourishing field which has already resulted in immensely valuable techniques such as the production of human insulin and novel approaches for the treatment of cancers. It is probably fair to state that the entire range of our current understanding - from normal cell function to disease states - will be dwarfed by advances in this field every few years.

Bacterial culturing and fermentation

Bacteria and other single-celled microorganisms require a certain set of conditions in order to grow and reproduce. These requirements vary from organism to organism. Around the turn of the century, biologists began to identify the nutrients, temperature and other environmental conditions which optimize bacterial growth. In a less scientific but extremely important context, the use of yeast in the production of alcoholic beverages, vintners and brewmeisters had perfected distinctive expressions of their art over the ages by carefully choosing appropriate yeasts and fermentation conditions, passing along their secrets from generation to generation. Even in the vineyard, for many centuries, enologists mastered some simple biotechnology with the ability to recognize and prevent disease-causing fungi and bacteria from infecting wine

grapes. They could instead encourage the growth of those fungi that imparted characteristic flavor to their vintages.

Fermentation is any of a set of chemical reactions which results in the breakdown of energy rich chemicals (such as sugar) into other products (such as alcohol). The reactions are usually carried out by a microorganism such as yeast (a fungus) or bacteria. *Fermenters* or *bioreactors* are the enclosures where fermentation takes place. These can, but need not be specialized, high quality stainless steel vessels. (Animal digestive tracts, for example, are in fact, fermenters.) Modern specialized fermentation vessels (also known as "reactor vessels") may be made of a panoply of materials depending on the application, and may vary in capacity from a few tenths of liter to many thousands of liters, depending on the desired quantity of bacterial or other microbial product. The design of modern vessels may allow them to tolerate high pressures and temperatures, and may in addition be configured to permit rapid switching of the culture medium to allow the growth of many different micro-organisms.

Both fermenters and bioreactors serve a single purpose: to provide a controlled environment in which living cells can grow - from simple bacteria and yeast cells to more complex plant, insect, and mammalian cells. In large measure the latter cells are much more fragile than bacteria and require an environment where stirring and heating can be very carefully controlled and measured.

Recent advances in the design, operation, and control of fermenters and growth media have increased product yields dramatically. These include:

o New designs for agitation and aeration to eliminate shear stresses in the fluid and to force the fluid to flow radially for improved distribution of oxygen and nutrients.

o New probes to measure and computer-control such critical parameters as temperature, acidity, dissolved oxygen and carbon dioxide.

o Novel design to enhance maintenance of sterility.

o Growth of mammalian and other cells on microspheres, hollow fibers and small capsules to circumvent damage from shear forces.

Fermenters and bioreactors are used in commercial biotechnology facilities which employ a variety of systems to encourage the growth of cells. These systems are generally divided into **batch** or **continuous** cultures. In the batch process, microorganisms consume the nutrients available in the culture broth as they secrete their product(s). The desired products are then separated from the culture medium and the surviving organisms are either recycled into fresh

medium or are inactivated and disposed of as waste. In continuous processes, fresh nutrients are continually introduced into the reactor vessel and depleted medium is removed along with bacterial products which are purified from the solution. Batch processes are easier to operate than the continuous procedures, but the latter can provide much more product over a given period of time. Most fermenters are steam sterilizable and therefore are pressure-rated vessels. The sterilization system may be a permanent fixture of the plant or may be a mobile unit attachable to a particular vessel or production line.

In the United States, the characteristics of the materials used and produced, including toxicity, determine safety and containment practices at a given facility, based on national and local laws. When a toxic product is involved, for example in the manufacture of diphtheria toxin for vaccine, discharge from the reactor vessel is inactivated with high temperature and chemical bleach. Frequent disinfection of equipment is normally carried out, as well as sampling of the environment for microbial contamination. Access to the fermentation vessel site is often restricted to those individuals directly involved with the work in the area. Filtered air supplies are a common characteristic of fermentation areas to prevent contamination of the cultures with unwanted microbes. If containment is a priority due to the pathogenic nature of the organisms in laboratory culture, negative air pressure may sometimes be required. Containment considerations also mandate the presence of a sump with sufficient capacity to contain a spill of fermenter contents.

Bacterial and other microbial cultures can be transferred from one reactor vessel to another, and this generally takes places via aseptic connections. In the United States, levels of containment are described in two documents: (1) "Biosafety in Microbiological and Biomedical Laboratories" and (2) "National Institutes of Health Guidelines for Research involving Recombinant DNA Molecules".

In some circumstances, bacteria themselves are the desired end product, rather than specific chemicals. For example, a strain of the Pseudomonas bacteria which produces a natural "anti-freeze" is undergoing field testing for application to crops in tests designed to prevent frost damage.

Thus, naturally occurring, microbially produced compounds, are finding ever increasing commercial utility. In addition, it is possible to induce bacteria to produce proteins and other materials usually made by other types of cells, including plant and mammalian cells (see section on gene manipulation below). In large commercial application, many tens of tons of bacteria may be grown daily which are capable of producing thousands of pounds of useful end-products.

Complex organisms and animals in biotechnology

Since the 1920s, animals have served as a source of valuable blood-borne proteins useful for the diagnosis and treatment of human disease. Most of these proteins are *antibodies* which react with, and neutralize the bacterial toxins and viruses, or the offending organisms themselves.

In the pre-antibiotic era, horse sera of various types were used for the treatment of pneumonia, diphtheria, tetanus and other serious infectious diseases. From patients ill with infection, bacteria were isolated, taken to the hospital laboratory and tested against a large panel of the horse sera available in the laboratory refrigerator. If a given serum appeared to neutralize the bacteria on a microscope slide, the serum was injected into the patient. Occasionally, cures were dramatic, but the treatment was less than optimal. Patients often had allergic reactions to the horse serum itself, or had infections for which no horse antibody could be found.

Though antibiotics have largely replaced horse (and other animal) sera as treatment for infectious diseases, animal antibodies are still useful for diagnosis and occasionally for treatment. Anti-toxins, which are animal antibodies directed against the protein products of some bacteria, are still used today in the treatment of tetanus and botulism in humans, and in veterinary medical treatment. Specific animal-derived antibodies are used routinely in the diagnosis of infectious disease and connective tissue diseases (such as lupus and rheumatoid arthritis) because they can actually localize the human antibodies that are markers for these illnesses. Further, the antibody responses of animals against various diseases serve as critically important models for understanding the pathogenesis and control of many medical disorders.

The DNA of some species of animals has been manipulated to permit the production of hormones and medications, and to enhance the growth and disease resistance of the animals themselves. Blood clotting factors (for the treatment of hemophilia and related diseases), medications to dissolve unwanted clots in heart attack victims , and growth hormone are just a small portion of the many important products now available from genetically altered animals and animal cells.

Applications of genetic manipulation in industry

Modern techniques of *gene cloning* and *gene splicing* (sometimes also referred to as *genetic engineering*) involve the introduction of a foreign gene into a bacterium or other cellular host. Genes are the molecular blueprints for proteins and peptides, and the goal of splicing is to persuade the new cellular host to accept the foreign gene, to multiply and to then manufacture or *express* the new

product. In this way, a large variety of pharmaceutical, veterinary, and agricultural products are being mass-produced today by bacteria in large fermenters.

Through the early 1970s, bacteria, fungi and other cells were used to manufacture only a limited number of products of commercial interest. These products were the naturally occurring materials produced by the organisms, which were then isolated from the fermentation broth and chemically altered, usually for vaccine production. In some circumstances, these organisms had demanding growth requirements which led to complex, expensive schemes for growing the organism and isolating the final product.

Bacteriologists had observed that a group of viruses which attacked bacteria, called *bacteriophages* , existed in the environment and multiplied within the bacterial cell, often causing the bacteria to rupture releasing huge numbers of new virus particles which could then go on to infect other bacteria. During their reproduction and spread to other bacteria, these virus particles would often carry pieces of the DNA from the bacterial cells where they had multiplied. As a result, genetic characteristics of one bacterium could be spread to another. Scientists exploited this natural phenomenon, and used the bacteriophages to transfer specific pieces of DNA from one bacterium to the next. For example, it became possible to remove the gene which directed the production of toxin in bacterial species with exacting growth requirements and insert it into the DNA of a bacterial species which grew easily in commonly available culture media.

Viruses were later discovered which could accomplish the same result with genes from plants, fungi, and even mammalian cells. Thus, it is now possible to remove a specific gene from a bacterial cell and place it in a plant cell (or vice versa). Similarly, genes of human DNA can be transferred into bacterial cells, fungi, or into other mammalian cells. Species of plants, bacteria, or animal possessing transplanted genes are referred to as *recombinant species.*

Today, biopharmaceutical facilities use genetically modified bacteria to produce a wide range of medical products. Most of the insulin used by diabetics in the United States is produced by a common, easy to grow intestinal bacterium called Eschericia Coli (E. Coli) into which the human DNA for insulin synthesis has been inserted. In addition to providing an easily controlled, high quality source of insulin, the E. Coli makes possible the use of actual human insulin, which is often much more effective than the previously employed insulin isolated from the pancreas glands of pigs and cows. Many vaccines against viral and bacterial diseases are produced from recombinant bacteria. Examples include vaccines for hepatitis B, diphtheria, tetanus, and pertussis.

Bioagriculture facilities are now able to produce disease-resistant plants using similar techniques. The Colorado potato beetle, which is responsible for millions of dollars of agricultural damage each year, is inhibited or killed by a protein made by the bacteria B. thurengensis (BT). For a number of years, farmers and gardeners alike have employed this bacterium or its protein product as an insecticide - a process which is limited by the high cost of spreading this naturally occurring insecticide on large fields. It is now possible to insert the gene for the B. thurengensis protein directly into developing plant cells, which results in a potato plant (and other plant species) which produce the insecticide as part of their normal metabolism. The BT protein directly damages the absorptive cells which line the intestinal tract of some harmful insects. The plants are no longer damaged by the Colorado potato beetle and many other insect pests, and crop yields are dramatically higher. Research continues into isolating additional disease-resistance genes that will permit plants to survive other infestations. The use of plant viruses, which offer the possibility of spreading selected, desirable genetic characteristics from plant cell to plant cell is one particularly exciting area of work.

This list of valuable products realized from genetic engineering technology continues to grow rapidly. In human and veterinary pharmaceuticals, food production, and in research to advance understanding of cell biology and metabolism, the role of genetic engineering will doubtless continue to grow.

Of great interest at this time are enzymes or whole bacteria which are capable of degrading complex chemical molecules, even toxic molecules such as methylene chloride or trichloroethylene, into innocuous element of carbon, hydrogen, and oxygen, thereby destroying a wide variety of hazardous waste. Some bacteria have been isolated from natural sources while others may employ genetic engineering technology to perform "bioremediation" of toxic and hazardous waste. This field is growing rapidly since classical destruction methods such as incineration and landfilling become prohibitively more expense and new sites become more difficult to locate.

Catalytic antibodies from cells

Cells from many species can be employed to produce compounds of interest and utility. In yet another exciting application of this technology, antibodies from mammalian cells are being used to accelerate industrially important chemical reactions.

In the chemical industry, engineers are constantly searching for compounds which catalyze or increase the rate of the chemical reactions that lead to a desired product. Typically, the economic viability of the reaction sequence depends on the presence of catalysts to overcome unusually severe conditions otherwise required to permit reactions to proceed. Unfortunately, finding these

catalysts, or synthesizing them, is rarely possible. On rare occasions, biochemists were able to identify bacterial enzymes which acted as catalysts for the reactions they were interested in accelerating.

The equivalent of "designer" enzymes has been realized from an unexpected source: mammalian white blood cells which normally produce antibody. It is now possible to find antibodies which bind to the precursors of a desired chemical end product and which further force these precursors to react. These *catalytic antibodies* permit chemists to carry out complex, previously impossible chemical reactions under simplified conditions, and turn out large quantities of formerly expensive chemical products. The white blood cells making the antibodies can be cloned and kept as part of a "library" of cells which are continuously available as sources of engineered enzymes. Entire classes of reactions, which for decades could only be carried out at slow speeds or under harsh conditions (such as high temperature, pressure or acidity) can now be carried out with few, if any, demanding requirements. Examples include the synthesis of complex food additives and proteins.

Microencapsulation

Although not included in a strict definition of biotechnology, microencapsulation is a complementary industrial process that has many applications. The term refers to the approaches and techniques used to surround very small amounts of biological or other materials with a specialized coating.

The wall of a microcapsule can function to protect the interior core material (e.g. a bacteria or drug) from hostile environments, or to release the core material under a specific stimulus such as heat, pressure, or light. This release can be at a constant, finite rate, or can be arranged to be instantaneous after the proper stimulus is applied. Microencapsulation technology is mature and conceptually straightforward. It is used in many industries around the world. Everyday applications include: "carbonless" pressure sensitive paper; scratch-and-sniff perfume advertisements in magazines; adhesive; preservation of materials and additives of high potency, fragility or value as foods (such as flavors and vitamins); controlled release of pesticides and herbicides; and countless uses in the pharmaceutical industry to target and release drugs. Wall materials come from a wide variety of sources (e.g. plant derivatives, synthetic polymers and synthetic gums) and can be engineered to provide a wide repertoire of properties such as permeability, solubility, hardness and stickiness.

Summary

Biotechnology is a rapidly changing science. Compared to the rather crude techniques in the 1970s, the advances of the past two decades are truly staggering. Gene transfer technology, which began with the modification of small pieces of DNA in simple cells like bacteria is now on the doorstep of the ultimate challenge - the replacement of defective genes in inherited or acquired human illness. Hemophilia, sickle cell anemia, congenital immune system disorders, and cystic fibrosis - among many others - are all genetically based diseases theoretically amenable to correction by gene transfer. Starting in early 1992, patients with cystic fibrosis are being purposefully exposed to partially inactivated viruses which will transfer a healthy gene into the cells of their lungs to replace the defective genetic material they inherited.

In agriculture, industrial chemistry and research, the tools of biotechnology have resulted in disease-resistant plants and highly efficient synthetic pathways to replace older, costly production techniques. Catalytic antibodies promise far more efficient, economical synthesis of complex chemicals. Indeed, the production of several commercial products such as artificial sweeteners is already being effected by this technology.

Geneticists, microbiologists, and agricultural specialists are intensively working to catalog the immense number of genes from cells of diverse species in the hope of identifying new techniques and applications of existing techniques to exploit biological processes. An effort to characterize the entirety of the human genome has begun, planned for completion over the next decade. There is little doubt that a revolution in our understanding of cell biology is underway, and that we have only begun to realize its immense potential benefit.