Overview of Biotechnology

Deependra Dhakal

Academic year 2019-2020

Background

Overview

The U.S. Department of Agriculture (USDA)'s classical interpretation

Agricultural biotechnology is a collection of scientific techniques, including genetic engineering, that are used to create, improve, or modify plants, animals, and microorganisms...

- Agricultural Research Service (ARS), the in-house research agency of USDA, classifies biotechnology research into six components:
  - basic engineering of recombinant DNA;
  - DNA sequencing;
  - genomic mapping with molecular markers;
  - monoclonal antibodies;
  - cell fusion and chromosome transfer;
  - biologically-based processing

Overview

- Humans have continually improved crop plants and animals by selective breeding, mostly by trial and error.
- However, the field of genetics is quickly transitting into a highly advanced scientic practice, wherein there is now not much place for trial and error studies.
- The modern biotechnology of interest centers upon the newfound ability to remove DNA from cells of an organism, modify it, and reinsert it into cells where it will be functional.
- This process is sometimes called "genetic engineering," and products therefrom have often been ingenuously termed "genetically modified organisms" (GMOs), even though all crop plants are genetically modified in one way or another.
- The traditional breeder's available gene pool is predominantly limited to those genes in sexually-compatible organisms, whereas modern biotechnology enables some new, wider-ranging, choices.

Classical plant breeding

- Practicing classical plant breeding means many thousands of plants must be cross-pollinated to find the one offspring with higher yield.
- In crossing plants,
  - Pollen must be taken from one plant and manually placed on another.
  - The possibility of finding improved traits is limited by the amount of genetic diversity already present in the plants.
  - Consequently, if the two plants that are crossed share many of the same genes, the amount of possible improvement is limited.
- Therefore, scientists have searched for better ways to improve plants.

Mutation breeding

- In the 1920s, scientists realized that *mutations* could be induced in seeds by using chemical mutagens or by exposure to X-rays or gamma rays.
- Outcome of such treatments is even less predictable than traditional breeding methods.
- Successful in world of flowers; new colors and more petals have been expressed in flowers such as tulips, snapdragons, roses, chrysanthemums, and many others.
- Mutation breeding has also been tried on vegetables, fruits, and crops. For instance, peppermint plants that are resistant to fungus were generated this way.

Modern breeding

- Introduction of molecular breeding– more predictable way to enhance crops.
- Movement of genes from foreign sources into a specific plant, resulting in a *transgenic* plant.
- The foreign gene, or transgene, may confer specific resistance to an insect, protect the plant against a specific herbicide, or enhance the vitamin content of the crop.
- With something so powerful as genetic engineering, one mistake could have profound and wide-ranging effects. We must impose tough controls on the genetics supply industry and work to make sure that the new techniques are in the service of the global community

History

History

- While some were seeking to alter the genetic make-up of living things by transferring specific genes from one organism to another, they now had tools to alter exactly the hereditary material at the molecular level.
- Walter Gilbert carried out the first recombinant DNA experiments in 1973
- First hybridomas created in 1975
- The production of monoclonal antibodies for diagnostics was carried out in 1982,
- The first recombinant human therapeutic protein, insulin (humulin), was produced in 1982.
- In 1976, the U.S. company Genentech became the first biotech company to develop technologies to rearrange DNA.
- 1980 ruling of the U.S. Supreme Court allowed genetically-engineered microorganisms to be patented. This means that virtually any lifeform on this planet can theoretically become the private property of the company or person who "creates" it.

History

- Clues to understanding fermentation emerged in the seventeenth century when Dutch experimentalist Anton Van Leeuwenhoek discovered microorganisms using his microscope.
- He unraveled the chemical basis of the process of fermentation using analytical techniques for the estimation of carbon dioxide.
- Two centuries later, in 1857, a French scientist Louis Pasteur published his first report on lactic acid formation from sugar by fermentation. He proved that fermentation is the consequence of anaerobic life and identified three of its types:
    - Fermentation, which generates gas;
    - Fermentation that results in alcohol; and
    - Fermentation, which results in acids.

History

- At the end of the nineteenth century, Eduard Buchner observed the formation of ethanol and carbon dioxide when cell-free extract of yeast was added to an aqueous solution of sugars. Thus, he proved that cells are not essential for the fermentation process and the components responsible for the process are dissolved in the extract. He named that substance "Zymase".
- During World war I,
  - Germany produced glycerine for making the explosive nitroglycerine
  - Bacteria that converts corn or molasses into acetone for making the explosive cordite.
  - Sir Alexander Fleming's discovery of penicillin, the first antibiotic, proved highly successful in treating wounded soldiers.

Transgenic technology vs traditional breeding

- A plant can be transformed with a gene from any source, including animals, bacteria, or viruses as well as other plants, whereas traditional cross-breeding methods move genes only between members of a particular genus of plants.
- Furthermore, transgenes can be placed in precise locations within the genome and have known functions that have been evaluated extensively before being inserted into the plant.
- In traditional breeding, on the other hand, the identity of genes responsible for improving the crop is rarely known.

Timeline I

Table 1: History of biotechnology

Date	Event
5000 BC	Indus and Indo-Aryan civilizations practiced biotechnology to produce fermented foods and medicines and to keep the environment clean.
4000 BC	Egyptians used yeasts to make wine and bread.
1750 BC	The Sumerians brewed beer.
250 BC	The Greeks used crop rotation to maximize crop fertility.
1500 AD	The Aztecs made cake from spirulina.
1663 AD	Robert Hook first described cells.
1675 AD	Microbes were first described by Anton Van Leeuwenhock.
1859 AD	Darwin published his theory of evolution in 'The Origin of Species.'
1866 AD	Gregor John Mendel published the basic laws of genetics.
1869 AD	DNA was isolated by Friederich Miescher.
1910 AD	Genes were discovered to be present in chromosomes.
1917 AD	The term 'biotechnology' was used to describe fermentation technology.
1928 AD	The first antibiotic, penicillin, was discovered by Alexander Flemming.
1941 AD	The term 'genetic engineering' was first used.
1944 AD	Hereditary material was identified as DNA.
1953 AD	Watson and Crick proposed the double helix structure of DNA.

Timeline

Table 2: History of biotechnology (...continued)

Date	Event
1961 AD	Deciphering of genetic code by M.Nirenberg and H.G. Khorana.
1969 AD	The first gene was isolated.
1973 AD	The first genetic engineering experiment was carried out by Walter Gilbert.
1975 AD	Creation of the first hybridomas.
1976 AD	The first biotech company.
1978 AD	World's first 'test-tube baby,' Louise Brown, was born through in vitro fertilization.
1981 AD	The first gene was synthesized. The first DNA synthesizer was developed.
1982 AD	The first genetically engineered drug, human insulin, produced by bacteria, was manufactured and marketed by a U.S. company. Production of the first monoclonal antibodies for diagnostics.
1983 AD	The first transgenic plant was created—a petunia plant was genetically engineered to be resistant to kanamycin, an antibiotic.
1983 AD	The chromosomal location of the gene responsible for the genetic disorder, Huntington's disease, was discovered leading to the development of genetic screening test.
1985 AD	DNA fingerprinting was first used in a criminal investigation.
1986 AD	The first field tests of genetically-engineered plants (tobacco) were conducted.
1990 AD	Chymosin, an enzyme used in cheese making, became the first product of genetic engineering to be introduced into the food supply
1990 AD	Human genome project was launched.
1990 AD	The first human gene therapy trial was performed on a four-year-old girl with an immune disorder.
1991 AD	The gene implicated in the inherited form of breast cancer was discovered

Timeline

Table 3: History of biotechnology (...continued)

Date	Event
1992 AD	Techniques for testing embryos for inherited diseases were developed
1994 AD	First commercial approval for transgenic plant by the U.S. government.
1995 AD	First successful xenotransplantation trial was conducted, transplanting a heart from a genetically-engineered pig into a baboon.
1996 AD	First commercial introduction of a 'gene chip' designed to rapidly detect variances in the HIV virus and select the best drug treatment for patients.
1996 AD	Dolly, the sheep was cloned from a cell of an adult sheep.
1998 AD	Embryonic stem cells were grown successfully, opening new doors to cell- or tissue-based therapies.
1999 AD	A U.S. company announced the successful cloning of human embryonic cells from an adult skin cell.
1999 AD	Chinese scientists cloned a giant panda embryo.
1999 AD	Indian scientists and companies started producing recombinant vaccines, hormones, and other drugs.
1999 AD	The draft of human genome sequence was published.

Major cellular techniques in biology

- Microscopy
- Cell sorting
- Cell fractionation
- Cell-growth determination

Major genetic techniques

- Chromosomal techniques
- Mutagenic technique
- Recombination in bacteria (Recombination DNA technology)
    - Tools
    - Making Recombinant DNA
    - DNA library
    - Transgenics (Introduction of Recombinant DNA into host cells)
    - Identification of recombinants
    - Polymerase chain reaction
    - DNA probes
    - Hybridization techniques
    - DNA sequencing
    - Site-directed mutagenesis
- Pedigree analysis in humans
- DNA isolation and purification techniques
- Molecular markers, TILLING and ZFN technology in plants

Major applications of biotechnology

- Biological fuel generation
- Single-cell protein
- Sewage treatment
- Environmental biotechnology
- Medical biotechnology
- Agriculture and forest biotechnology
- Food and beverage biotechnology
- Safety in biotechnology

Biotechnology process

- In previous century, industries linked to the fermentation technology had grown tremendously because of the high demand for various chemicals such as ethanol, butanol, glycerine, acetone, etc.
- The advancement in fermentation process by its interaction with chemical engineering has given rise to a new area – the bioprocess technology.
- Large-scale production of proteins and enzymes can be carried out by applying bioprocess technology in fermentation.
- Processes to create large quantities of chemicals, antibiotics, proteins, and enzymes in an economical manner.
- Bioprocess technology includes media and buffer preparation, upstream processing and downstream processing.

Biotechnology process: Upstream processing

- Includes:
  - microorganism media (development of processes for aseptic treatment of substrates or raw materials with the microorganism or the biocatalyst)
  - substrate, and the correct chemical environment to carry out the required biochemical reactions to produce the product.
- Unit operations involved in upstream processing are: Milling, Mixing, Media preparation, Sterilization, Cooling, Heating.

Biotechnology process: Bioreactor

- Initial step: designing of the appropriate type of bioreactor or fermentor.
- Bioreactors are vessels in which raw materials are biologically converted into specific products, using microorganisms, plants, animals, or human cells or individual enzymes.
- Bioreactor supports the natural process of cells by trying to maintain their environment to provide optimum growth conditions by providing appropriate temperature, pH, substrates, salts, vitamins, and oxygen.
- In most of the bioreaction processes the substrate of the biotransformation and the carbon source of the organisms will be the same.

Biotechnology process: Bioreactor

- Unit operations involved in bioreactions are: Mixing, Handling of microorganisms, plant or animal cells, Inoculation of the cells, Heating and cooling.
- Bioreactors can be classified according to the type of biocatalysts and the type of bioreaction.
- The first classification is based on the type of biological agent used:
  - microbial fermentors or
  - enzyme (cell-free) reactors
- Further classification is possible based on biochemical reactions and process requirements.

Biotechnology process: Downstream processing

- recovery and purification of the required product from the growth medium through a set of separation and purification techniques.
- each stage in the overall separation procedure is strongly dependent on the history and quality of the biological production process.
- maximization of production can lead to great difficulties in downstreaming and recently more attention is being paid to overall process optimization.
- includes techniques such as filtration, centrifugation, sedimentation, various types of chromatographic techniques, electrophoresis, etc.

Biotechnology process

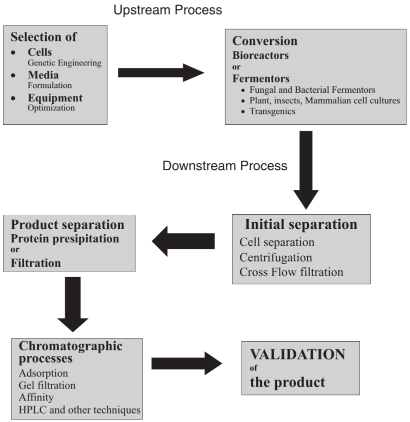

Figure 1: A typical biotechnology flow process

Industrial biotechnology

- Recombinant microorganisms, plant cells, and animal cells can be cultivated and used for large-scale production of industrially-important enzymes and chemicals. A list of such enzymes is given in Table 4

Table 4: Some major industrial enzymes and their sources and uses.

Enzymes	Sources	Uses
Amylases	Aspergillus niger, A. oryzae, B. licheniformis, B. subtilis, germinating cereals germinating barley	Hydrolyze starch to glucose, detergents, baked goods, milk cheese, fruit juice, digestive medicines, dental care
Invertases	Saccharomyces cerevisiae	Production of invert sugar, confectionery
Glucose isomerase	Arthrobacter globiformis, Actomoplanes missouriensis, Streptomyces solivaceus and E. coli	Conversion of glucose to fructose production of high fructose syrup, other beverages, and food
α D-Galactosidase	Mortierella vinacease	Raffinose hydrolysis
β D-Galactosidase	Aspergillus niger	Lactose hydrolysis
Papain	Papaya	Meat, beer, leather, textiles, pharmaceuticals, meat industry, digestive aid, dental hygiene, etc.
Proteases	Bacillus subtilis, B. licheniformis	Detergents, meat tenderizers, beer, cheese, flavor production
Pepsin	Hog (pig) stomachs	Cereals, pharmaceuticals
Trypsin	Hog and calf pancreases	Meat, pharmaceuticals
11-β-Hydroxylase	Curvularia lunata	Steroid conversion, bioconversion of organic chemicals
Ficin	Figs	Leather, meat, pharmaceuticals
Bromelain	Pineapple	Meat, beer, pharmaceuticals

Biotechnology product

- Traditional biotechnology products also include biologically-processed items like bread, cheese, and wine.
- Other modern biotechnologies, such as monoclonal antibodies or molecular markers as aids for traditional breeding and selection, are not relevant to genetic engineering but they constitute biotechnology products.

Fermentation

Introduction

- Decomposition of foodstuffs generally accompanied by the evolution of gas.
- The best-known example is alcoholic fermentation, in which sugar is converted into alcohol and carbon dioxide.
- This conversion, described by the equation below, was established by J. L. Gay-Lussac in 1815.

$$\underset{\text{Sugar}}{C_6H_{12}O_6} \rightarrow \underset{\text{Carbon dioxide}}{2CO_2} + \underset{\text{Alcohol}}{2C_2H_5OH}$$

History

- Before 1800 the association of yeast or leaven with fermentation had been noted, but the nature of these agents was not understood.
- Experiments of C. Cagniard-Latour, of F. T. Kutzing, and of T Schwann in 1837 indicated that yeast is a living organism and is the cause of fermentation.
- This view was opposed by such leading chemists as J. von Liebig and F. Wohler, who sought a chemical rather than a biological explanation of the process.
- The biological concept became generally accepted following the work of Louis Pasteur, who concluded that fermentation is a physiological counterpart of oxidation, and permits organisms to live and grow in the absence of air (anaerobically).

History

- This linked fermentation and putrefaction as comparable processes; both represent decompositions of organic matter brought about by microorganisms in the absence of air.
- The difference is determined by the nature of the decomposable material;
  - sugary substances generally yield products with pleasant odor and taste (fermentation),
  - proteins give rise to evil-smelling products (putrefaction).
- Pasteur also discovered the lactic acid and butyric acid fermentations, and from his experiments concluded that each kind of fermentation was caused by a specific microbe.
- Later work supported this idea to a large extent, and considerably increased the number of specific fermentations.

Process

During fermentation organic matter is decomposed in the absence of air (oxygen); hence, there is always an accumulation of reduction products, or incomplete oxidation products. Some of these products (for example, alcohol and lactic acid) are of importance to society, and fermentation has therefore been used for their manufacture on an industrial scale. With regard to historic roots of the process, Converting dry grains and other seeds into something more appetizing than a gruel must have made agriculture more attractive and valuable. Alcohol, despite its dangers, provided (and still provides), in reasonable moderation, a basis for social interaction. There are also many microbiological processes that go on in the presence of air while yielding incomplete oxidation products. Good examples are the formation of acetic acid (vinegar) from alcohol by vinegar bacteria, and of citric acid from ugar by certain molds (for example, *Aspergillus niger*). These microbial processes, too, have gained industrial importance, and are often referred to as fermentations, even though they do not conform to Pasteur's concept of fermentation as a decomposition in the absence of air.

Fermentation technology: Milk fermentation

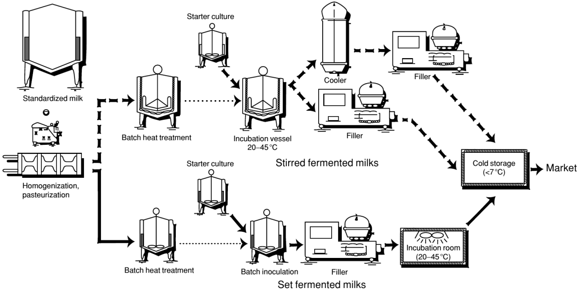

Figure 2: Basic steps in manufacture of fermented milks. From International Dairy Federation (1988) Fermented Milks-Science and Technology. International Dairy Federation Bulletin No. 227.

Bibliography

Further study

Also see: Nair (2008)

References

Nair, A Jayakumaran. 2008. *Introduction to Biotechnology and Genetic Engineering*. Laxmi Publications, Ltd.