The History of Biotechnology in Medicine and It's Future Perspectives

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Abstract

Bioprocess technology encompasses all of the basic and applied sciences in microbiology, biochemistry and molecular biology as well as the engineering aspects to fully exploit living systems and bring their products to the market place.

To-day bioprocesses have become widely used in several fields of commercial biotechnology in medicines, veterinary medicines, food ingredients, agriculture, environmental science and biological chemicals.

While our understanding of biotechnological process has rapidly and remarkably advanced in recent years, it has been in existence since prehistoric times, making it one of the oldest technology even before the discovery of microbiology.

The term of bioconversion that is also known by the name biotransformation refers to the use of living organisms or its extracted enzymes to carry out chemical reactions that are not feasible or costly when produced by synthetic chemistry methods. These living organisms convert a substance to a chemically modified form with multiple uses and applications.

Recent advances in the fields of molecular genetics and nucleic acid chemistry have resulted in a proliferation of biotechnology products. In medicine, modern biotechnology can be used to manufacture existing and new biomedicines relatively easily and cheaply. This modern biotechnology find application in biomedicines such monoclonal antibodies (mAbs), therapeutic proteins, pharmacogenomics, and genetic testing.

Keywords: Biotechnology; molecular biology; microbiology; biochemistry; enzymology; genetic engineering; enzymes immobilization; bioconversion; biotransformation; biopharmaceuticals; therapeutic proteins; viruses; biomedicine; molecular diagnostics

Abbreviations: FDA: Food and Drug Administration; AAV: Adeno-associate virus; DNA: Deoxy-riobo nucleic acid; PCR: Polymerase chain reaction; ELISA: Enzyme-linked immunosorbent assay

Introduction

Historical perspective

Biotechnology refers to the use of living organisms, or the metabolites produced by these organisms for wide ranges of applications. It is a field of science that is overlaps with industrial microbiology, biochemistry, molecular biology and bioengineering.

Biotechnology has been used for several thousand years, initially in the area of food production and preservation. Ancient Egyptian were brewing beer and producing baked products by the 4th millennium BC.

While our understanding of biotechnology has rapidly and remarkably advanced in recent years, biotechnology has been in existence since prehistoric times, making bioprocess technology one of the oldest science even before the discovery of the field of microbiology.

Historical Perspective of Biotechnology in Medicine

500 BC: In China, the first antibiotic, moldy soybean curds, is put to use to treat boils.

AD 100: The first insecticide is produced in China from powdered chrysanthemums.

1859: British scientist Darwin published the origin of species.

1877: French scientist Pasteur noted relation between microorganisms and infection diseases.

1881: German scientist Koch developed techniques for handling and maintenance of microbial cultures.

1894: Japanese scientist Takamine patented process to isolate diastase enzyme from mold.

1911: American pathologist Peyton Rous discovers the first cancer-causing virus.

1928: Scottish scientist Alexander Fleming discovers the antibiotic penicillin against Staphylococcus aureus.

1940: British scientists Florey and Chain, elucidated penicillin structure and its antibacterial properties against gram positive bacteria.

1940: American scientist Waxman discovered the antibiotic streptomycin against gram negative bacteria.

1942: Penicillin is mass-produced by fermentation for the first time.

1950: The first synthetic antibiotic is created.

1950: The production of cortisone for the first time.

1950: The production of polio and peruses vaccines for the first time.

1951: Artificial insemination of livestock is accomplished using frozen semen.

1958: DNA is made in a test tube for the first time.

1970: American scientists Kohler and Milstein developed the method for the development of monoclonal antibodies.

1978: Recombinant human insulin is produced for the first time.

1979: Human growth hormone is synthesized for the first time.

1980: Smallpox is globally eradicated following 20-year mass vaccination effort.

1980: The U.S. Supreme Court approves the principle of patenting organisms, which allows the Exxon oil company to patent an oil-eating microorganism.

1981: Scientists at Ohio University produce the first transgenic animals by transferring genes from other animals into mice.

1982: The first recombinant DNA vaccine for livestock is developed.

1982: The first biotech drug, human insulin produced in genetically modified bacteria, is approved by FDA. Genentech and Eli Lilly developed the product.

1985: Genetic markers are found for kidney disease and cystic fibrosis.

1986: The first recombinant vaccine for humans, a vaccine for hepatitis B, is approved.

1986: Interferon becomes the first anticancer drug produced through biotech.

1987: Development and production of the recombinant tissue plasminogen activator (tPa) used to dissolve blood clots in patients with acute myocardial infarction. Also used to treat non-hemorrhagic stroke.

1987: Development and production the interferon gamma 1b for the treatment of chronic granulomatous disease.

1990: The first successful gene therapy is performed on a 4-year-old girl suffering from an immune disorder.

1992: FDA approves bovine somatotropin (BST) for increased milk production in dairy cows.

1993: FDA approves Betaseron®, the first of several biotech products that have had a major impact on multiple sclerosis treatment.

1993: Development and production the recombinant somatropin a growth hormone for pre- kidney transplant operation.

1993: Development and production of the recombinant DNAse, the inhalation drug Pulmozyme (dornase alfa) for the treatment of patients with cystic fibrosis disease.

1994: The first breast cancer gene is discovered.

1994: The Americans are certified polio-free by the International Commission for the Certification of Polio Eradication.

1995: Gene therapy, immune-system modulation and the produced recombinant antibodies enter the clinic trials against cancer.

1996: A gene associated with Parkinson's disease is discovered.

1996: The first genetically engineered crop is commercialized.

1997: A sheep named Dolly in Scotland becomes the first animal cloned from an adult cell.

1998: FDA approves Herceptin®, a Pharmacogenomics breast cancer drug for patients whose cancer over expresses the HER2 receptor.

1999: A diagnostic test allows quick identification of Bovine Spongicorm Encephalopathy (BSE), also known as "mad cow" disease and Creutzfeldt - Jakob disease (CJD)

2000: - Development and production of the clot-busting drug TNKase for the treatment of acute myocardial infarction.

2003: Development and production of Anti-VEGF monoclonal antibody Avastin (bevacizumab) for the treatment of metastatic cancer of the colon and rectum.

2006: The recombinant vaccine Gardasil® was developed against human papillomavirus (HPV) and approved by FDA as the first preventative cancer vaccine.

2010: The first interleukin-6 (IL-6) receptor-inhibiting monoclonal antibody was -approved to treat rheumatoid arthritis (RA).

2013: The first Genentech antibody-drug conjugate (ADC) to receive FDA approval for the treatment of HER2-positive metastatic breast cancer.

2014: The first artificial mini-stomach (called gastric-organoid) made in lab from stem cells could spur pure research into cancer, ulcers, and diabetes.

Today, biotechnology has become widely used in several fields of commercial bio-products and bio-active ingredients in medicine, veterinary medicines, food processing, nutritional health, agriculture, and environment, plus for the production of biological chemicals such as enzymes, antibiotics, hormones, vitamins, amino acids, organic acids, therapeutic proteins etc.

The basic purification step such as distillation of ethanol was developed and applied in China in the 2nd millennium BC. This distillation process was the early knowledge on purification technology and was the first down stream process for industrial fermentation.

Today in addition to distillation, down stream processes utilizing immerging advanced separation technologies such as centrifugation, extraction, column separation, membrane technology, electro dialysis etc.

The modern biotechnology developed from 19th century after the discovery of microbiology and microbial life. This modern biotechnology is still based on the principle of combining living organisms with nutrient under controlled fermentation conditions to produce high yield of the desired end products.

In the first half of the 20th century world war I drove the development of the first large scale fermentation process outside old alcohol production to acetone, butanol and glycerol fermentation process for the production of explosives and citric acid fermentation process for the disruption of Italian citrus exports.

The pressure of World War II brought us the antibiotic fermentation in the form of penicillin [1] against Gram positive (G+) bacteria and then streptomycin [2] against Gram negative (G -) bacteria.

In the 1980s, the recombinant gene technology led to the development of genetically engineered *Escherichia coli* bacteria for the production synthetic humanized insulin [3] at relatively low cost for the treatment of diabetes. This was the first biomedicine manufactured with recombinant technology. Insulin was previously extracted from the pancreas of pigs or cattle.

This newly developed genetic engineering [4] and the newly developed monoclonal antibody [5] technology has led to the introduction of a large number of new biomedicines such as interferon [6], tissue plasmogen activator [7], erythropoietin [8], colony-stimulating factors [9], and monoclonal-antibodies [5]. In addition, this newly advanced molecular biology technology resulted in significant new bio products not only in medicine but in a wide broad applications such as but not limited to agriculture, food processing, nutritional health , bio fuels, biochemical's and environments.

Because biotechnology process uses living organisms, they offer several advantages over the conventional synthetic chemistry process. Biotechnology process can use renewable resources as raw materials and can operate at lower temperature, pressure and pH to produce high yield of bio products with less energy consumption and lower cost.

In the most of biotechnology process, enzymes in whole living organisms or extracted from living organisms cellular components are used to catalyze the biochemical reactions for the production of desired bio products that have applications in medicine, agriculture, foods, nutritional health, energy, chemical industry, environmental cleanup, etc.

Bio Conversion and Bio Transformation

Enzymes are catalyst that can convert raw materials as a substrate to form desired end product. These enzymes carry out the bioconversions or biotransformation for the production of target end products.

In the early discovery of bioconversion or biotransformation process, three major challenges were present that resolved by newly developed advanced technologies.

First Challenge

Maintaining the desired optimum conditions (temperature, pressure, pH, oxygen levels, etc) for the bioconversion or biotransformation process.

Solution: The development of automated and computerized equipments to assist in maintaining the desired optimum conditions for bioconversion/biotransformation process and monitoring the reactions. These instruments improved the bioconversion or biotransformation efficiency and increased end-products yield.

Second Challenge

The formation of unwanted impurities during the bioconversion or biotransformation process resulted in difficulties to produce the end product in pure forms.

Solution: The development of separation and purification techniques for down stream process resulted in methods to economically produce highly pure end products with higher recovery efficiency.

Third Challenge

The cost of catalytic enzymes used in the bioconversion or biotransformation process resulting in a higher production cost.

Solution: The developments of immobilized enzymes [10] and cells systems for either continuous and semi-continuous bioconversion or biotransformation process have dramatically reduced the production cost of end products.

In addition to enzymes application as a catalyst for bioconversion and biotransformation process, Enzymes application in medicines extended to: (1) Treat disorders like low blood pressure, myocardial infraction, tachycardia, and head or spinal injuries. One of such enzyme is Streptokinase. (2) Assist metabolism as an aid to digestive system. One of such enzyme is Papain (3) Assist drug delivery deeper to the target tissue. One of such enzyme is Hyaluronidase.

Genetic Engineering for Gene Therapy

Following early biotechnology advances in genetic engineering of bacteria, cells, and small animals, scientists started considering how this genetic engineering technology could be applied in medicine to modify human chromosome for the treatment of genetically related diseases. The two main approaches that have been considered are adding gene to replace a gene that wasn't working properly and disrupting genes that were not working properly.

The DNA delivery into cells can be accomplished by two major methods: Recombinant viruses (viral vectors) or Naked DNA (no-viral method).

Recombinant DNA (Viral Vector): Viruses bind to their specific target hosts and introduce their genetic material as part of their own replications into the host cell DNA. By replacing the viral DNA with a therapeutic DNA the virus became a vehicle to deliver the therapeutic DNA to the target cell as shown in figure 1.
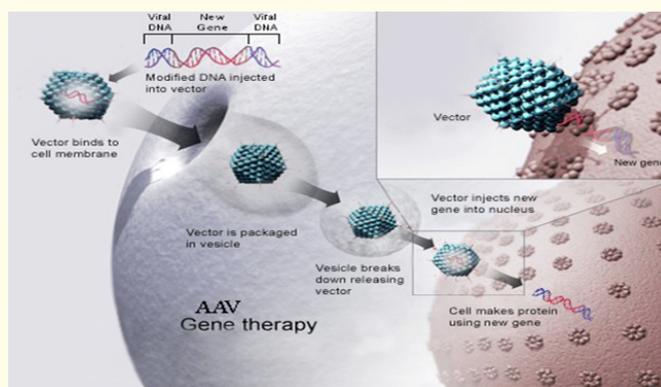

Figure 1: Adino Associate Virus (AAV) Gene Therapy. A new gene is inserted into a patient cell using the AAV protein shell. Once inside the nucleus, the new gene makes functional protein to treat a disease. http://commons.wikimedia.org/wiki/File:AAV_Gene_Therapy.jpg#/media/File:AAV_Gene_Therapy.jpg

Several viruses can be used for human gene therapy including *Retrovirus, Adenovirus, Lentivirus, Herpes simplex virus, Vaccinia virus* and *Adeno-associate virus.*

Naked DNA (non-viral): Non-viral method is direct injection (transfection) of a naked DNA into host cell. Transfection can be done by several methods such as electroporation or gene gun.

Diseases that are considered for evaluating by these two gene therapy approaches are the diseases that are caused by single gene defects [11], such as cystic fibrosis [12], hemophilia [13], muscular dystrophy [14], thalassemia [15] and sickle cell anemia [16].

Gene therapy approaches are still in the early stage of experimental technique, although in year 2012 Glybera® a drug for the treatment of a disease caused by a defect in a single gene for the enzyme lipoprotein lipase [15] became the first FDA approved gene therapy medicine for general population treatment in Europe and United States.

Gybera® drug is a recombinant *Adeno-associate virus* (AAV) as a viral vector delivers an intact copy of the human lipoprotein lipase (LPL) gene into muscle cells

Genetic engineering for therapeutic recombinant proteins

Therapeutic proteins or protein-based therapeutics are recombinant proteins that are genetically engineered in the laboratory for pharmaceutical applications. Insulin for diabetes was the first therapeutic protein developed by this advanced biotechnology.

Today, these Proteins-based therapeutic [17] are showing high recognition of their potential applications in medicine for treatments cancer, infection diseases, hemophilia, anemia, multiple sclerosis and hepatitis These protein-based therapeutics are divided into five groups based on pharmacological activities: (1) Replacing a protein that is deficient or abnormal. (2) Enhancing an existing pathway. (3) Providing a novel function or activity. (4) Interfering with a molecule or organisms. (5) Delivering other compounds or proteins, such as radionuclide, cytotoxic drug, or effectors protein.

Therapeutic recombinant proteins are produced by fermentation step as upstream process and recovery/purification step as a downstream process. Bacteria, yeast and mammalian cells are widely used as a host strains for these therapeutic recombinant proteins production. In the case of glycosylated recombinant therapeutic proteins mammalian cells are widely selected for the fermentation step.

The total sales of therapeutic recombinant proteins are over $100 billion and growing. The fastest growing therapeutic recombinant proteins are genetically engineered antibodies for cancer therapy. Antibodies support the immune response and on the other hand antibodies can specific target therapeutic agents to a tumor cell site through conjugation/fusion

Clinical Trials

It is very important to highlight that experimental drugs including bio medicines must go through *in vitro* testing in laboratory on animal or human cells, followed by multiple stages of *in vivo* testing on animals and human before the approval for the treatment of general population.

Clinical trials are referring to the *in vivo* testing of experimental drug on human. This human testing must be conducted and evaluated on a selected group of patients in hospital. Hospital's personnel are responsible to perform these clinical trials with good observation and precise documentations.

Before pharmaceutical companies start clinical trials on the experimental drug, they must first test the experimental drug *in vitro* in the laboratory on human or animal cells and must conduct extensive *in vivo* preclinical studies on animals for toxicity evaluation before ever initiating clinical trials.

Clinical trials are the responsibility of the pharmaceutical companies with hospital personnel. Both are responsible to follow the protocol and regulations that are established by the national regulatory authorities such as FDA in United States. These agencies are responsible to review the clinical trial data for disapproval or approval the experimental drug for commercialization general population treatment.

Clinical trials are commonly classified into four phases [17]. The data generated from each phase is treated as a separate clinical trial and must go through evaluation and approval process before initiating the second phase of the clinical trial.

These four clinical trials phases are

Phase (0) Trial

This is the first experimental drug testing on human trials for its safety. Single sub therapeutic doses of the experimental drug are given to 10 to 15 healthy people to generate preliminary data on the experimental drug's pharmacodynamics to evaluate the effect of the experimental drug on the human body and pharmacokinetics to evaluate the effect of human body on the experimental drug.

Phase (1) Trial: Testing the experimental drug on a small group of healthy people to evaluate its safety, the safe dose range, and identify its side effects.

Phase (2) Trial: The experimental drug is given to group of selected patients (100-300) to see if it is effective and for further evaluation of its safety against placebo.

Phase (3) Trial: This is the final confirmation on safety and efficacy against placebo. The experimental drug is given to large groups of patients (1,000-3,000) to confirm its effectiveness, monitor its side effects, and compare it to the safety of commonly used marketed medicines on general population for treatment.

If the experimental drug is successfully passes through the above four phases it will be approved by the national regulatory authority such as FDA in United States for Commercialization as new medicine to treat general population.

Phase (4) Trial: This is the post marketing studies after the approval of the newly discovered medicine for commercialization. This phase is mainly to generate additional information from general population treated with the new medicine to fully understand the treatment's risks, benefits, and optimal use.

The time required for new medicine-development process is about 10-15 years. It takes the average of 6 years for both drug discovery and laboratory testing and the average of 8 years for these four clinical trial phases.

Molecular Diagnostics

The discovery of genes, DNA sequencing and genes expression for protein synthesis led to the development and the application of molecular diagnostics in medicine.

Molecular diagnostics is a technique used to detect biological markers in the genome and proteome. The technique offered the prospect of personalized medicine [18] utilizing biological assays such as PCR (Figure 2), ELISA (Figure 3), and fluorescence *in situ* hybridization for assay and for detection the expression patterns of genes and proteins utilizing detection methods such as mass spectrometry and gene chips (Figure 4).
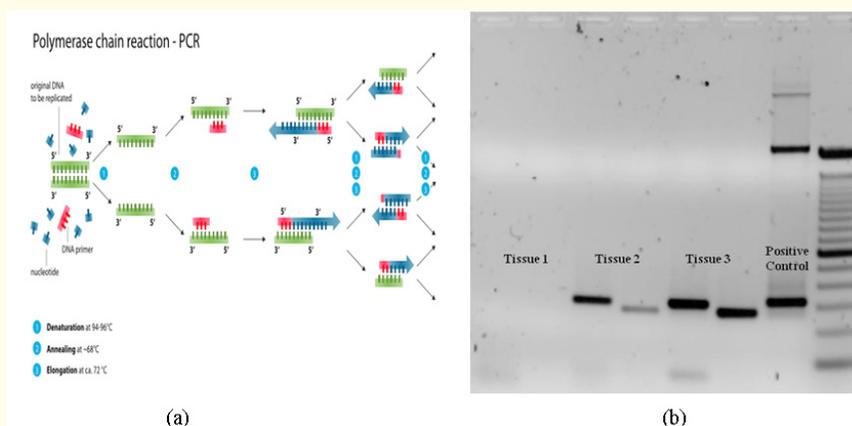

Figure 2: a) PCR chain reaction, b) Gel electrophoresis for PCR products stained by Ethidium bromide for detection.
http://commons.wikimedia.org/wiki/File:Roland_Gel.JPG#/media/File:Roland_Gel.JPG

Molecular genetics are very useful with a wide range of applications in medical testing such as cancer, infection diseases, parental, human leukocyte antigen, pharmacogenomics, and for disease risk managements
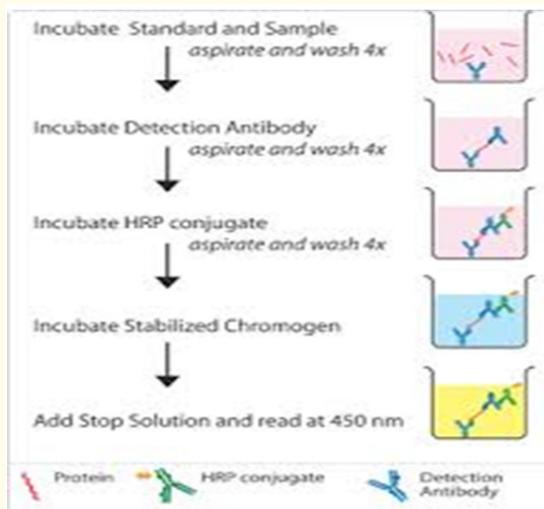
Figure 3: Schematic of General ELISA procedure Life Technologies-Inverogen.
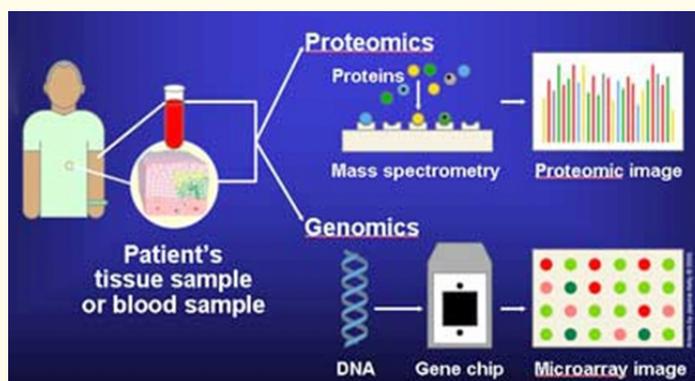
Figure 4: Molecular Diagnostics, Molecular diagnostics uses technique such as mass spectrometry and gene chips to capture the expression pattern of proteins and genes.
http://www.cancer.gov/cancertopics/understandingcancer/moleculardiagnostics/AllPages

US Food and Drug Administration (FDA) regulate and provide guidance for marketing *in vitro* diagnostic devices (IVD) including molecular diagnostics. There are three regulatory paths [19] for obtaining FDA approval for diagnostic devices: (1) the new diagnostic test is equivalent to an existing diagnostic test in the market, then the 510(k) is the regulatory path for the approval, (2) the new diagnostic test is not equivalent to any diagnostic test in market and is critical in making medical decision for diagnostic and treatment, then the pre-market approval (PMA) is the regulatory path for the approval, (3) the new diagnostic test is not equivalent to any diagnostic test in market but has low or moderate risk in making medical decision for diagnostic or treatment, then the diagnostic test can be marketed under "home brew" guideline.

Future perspectives of biotechnology in medicine

Today, the biotechnology has reached a critical size based on a broad understanding of genomics [20], proteomic [21], bioinformatics [22], genetic transformation [23] and molecular breading [24]. These advanced technologies are waiting to be transferred to a large scale bioprocess operations.

Genetic engineering stimulated hopes for both therapeutic protein drugs and for biological organisms themselves, such as modified human cells for treatment of genetic diseases. It is expecting that genetically engineered hosts such as insects, plant cells, and transgenic plants or animals, sooner or later will reach much broader applications, including the production of new cancer treatment drugs such as therapeutic proteins [25] and protein fusion [26] as specific target for tumor cells. DNA sequences [27] is a contributing factor for drug development and is a great information on human genomic that will assist researchers to identify mutations in tumor cells and to evaluate the effect of such mutations on cancer cells sensitivity to certain cancer drugs.

In addition, the use of extreme-thermopiles microbes [28] in recombinant technology to develop unique enzymes active at high temperature as biocatalysts in non-aqueous solution will broaden the technology platform to produce huge reservoir of new bio medicines and bio products with wide applications in medicine, food, agriculture, environment, energy and chemical industry.

Conclusion

Finally, now a day, the practice of medicine has changed dramatically over the years through pioneering advanced in biotechnology research and innovation. This current advanced biotechnology helping millions of patients worldwide to benefit from therapeutics drugs developed by pharmaceutical companies utilizing this advanced biotechnology in discovering and delivering innovative medicines to treat grievous illnesses.

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