BIOTECHNOLOGY

Dr. Amjad A. Mohammed **Third class / Biology**

Lec.( )

What is biotechnology?

Biotechnology is a combination of two individual terms: Biology and Technology.

The wide concept of "biotech" or "biotechnology" include a wide range of procedures for modifying living organisms according to human purposes, going back to domestication of animals, cultivation of plants, and "improvements" to these through breeding programs using artificial selection and hybridization. Modern usage also includes genetic engineering as well as cell and tissue culture technologies.

Old Biotechnology is the one which involves the use of natural capabilities of microbes or cellular components for manufacture of useful products.

New Biotechnology involves the use of recombinant DNA technology, enzyme engineering, genetic engineering, etc., for developing improved capabilities of biological agents for production of beneficial products.

Definitions

According to United States, National Science Academy, **biotechnology** is the "controlled use of biological agents like cells or cellular components for beneficial use". It covers both classical as well as modern biotechnology.

More generally, **biotechnology** can be defined as "the use of living organisms, cells or cellular components for the production of compounds or genetic improvement of living things for the benefit of man".

As per **European Federation of Biotechnology**, Biotechnology is the integration of natural science and organisms.

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History of biotechnology

The history ,mainly, can be divided into three stages:

1- **Ancient biotechnology**: includes plant cultivation and animal domestication. All which related to food.

2- **Classical biotechnology**: built on ancient biotechnology, fermentation promoted food production and medicine

3- **Modern biotechnology**: manipulates of genetic information in organism, genetic engineering

For thousands of years, humankind has used biotechnology in agriculture, food production, and medicine. The term is largely believed to have been coined in 1917 by Hungarian engineer Károly Ereky. In the late 20th and early 21st century, biotechnology has expanded to include new and variety sciences such as genomics, recombinant gene techniques, applied immunology, and development of pharmaceutical and diagnostic tests.

- ✓ Agriculture has been dominant way of producing food since the ancient age.
- ✓ **The fermentation of beer processes were introduced in early Egypt, China and India, and still use the same basic biological methods. In this process, carbohydrates in the grains were broken down into alcohols such as ethanol. Later other cultures produced the process of lactic acid fermentation which allowed the fermentation and protection of other forms of food, such as soy sauce. Fermentation was also used in this time period to produce bread. Although the process of fermentation was not fully understood until Louis Pasteur's work in 1857.**

- ✓ In the early twentieth century scientists gained a greater understanding of microbiology and explored ways of manufacturing specific products.
- ✓ **In 1917, Chaim first used a pure microbiological culture in an industrial process to produce acetone, which the United Kingdom needed to manufacture explosives during World War I.**
- ✓ **Biotechnology has also led to the development of antibiotics. In 1928, Alexander Fleming discovered the mold *Penicillium*.**
- ✓ Ernst Boris Chain and Norman Heatley - to form what we today know as penicillin. In 1940, penicillin became available for medicinal use to treat bacterial infections in humans.
- ✓ The field of modern biotechnology is generally thought of as having been born in 1971 when Paul Berg's (Stanford) experiments in gene splicing had early success.
- ✓ Herbert W. Boyer and Stanley N. Cohen significantly advanced the new technology in 1972 by transferring genetic material into a bacterium,
- ✓ The commercial viability of a biotechnology industry was significantly expanded on 1980.

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Milestones in the History of Biotechnology

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	Robert Hook first described cells.
1675	Microbes were first described by Anton Van Leeuwenhock.
1859	Darwin published his theory of evolution in *'The Origin of Species.'*
1866	Gregor John Mendel published the basic laws of genetics.
1869	DNA was isolated by Friederich Miescher.
1910	Genes were discovered to be present in chromosomes.
1917	The term 'biotechnology' was used to describe fermentation technology.
1928	The first antibiotic, penicillin, was discovered by Alexander Flemming.
1941	The term 'genetic engineering' was first used.
1944	Hereditary material was identified as DNA.
1953	Watson and Crick proposed the double helix structure of DNA.
1961	Deciphering of genetic code by M.Nirenberg and H.G. Khorana.
1969	The first gene was isolated.
1973	The first genetic engineering experiment was carried out by Walter Gilbert.
1975	Creation of the first hybridomas.
1976	The first biotech company.
1978	World's first 'test-tube baby,' Louise Brown, was born through *in vitro* fertilization.
1981	The first gene was synthesized. The first DNA synthesizer was developed.
1982	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	The first transgenic plant was created—a petunia plant was genetically engineered to be resistant to kanamycin, an antibiotic.
1983	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	DNA fingerprinting was first used in a criminal investigation.
1986	The first field tests of genetically-engineered plants (tobacco) were conducted.
1990	Chymosin, an enzyme used in cheese making, became the first product of genetic engineering to be introduced into the food supply.
1990	Human genome project was launched.
1990	The first human gene therapy trial was performed on a four-year-old girl with an immune disorder.
1991	The gene implicated in the inherited form of breast cancer was discovered.
1992	Techniques for testing embryos for inherited diseases were developed.
1994	First commercial approval for transgenic plant by the U.S. government.
1995	First successful xenotransplantation trial was conducted, transplanting a heart from a genetically-engineered pig into a baboon.
1996	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	Dolly, the sheep was cloned from a cell of an adult sheep.
1998	Embryonic stem cells were grown successfully, opening new doors to cell- or tissue-based therapies.
1999	A U.S. company announced the successful cloning of human embryonic cells from an adult skin cell.
1999	Chinese scientists cloned a giant panda embryo.
1999	Indian scientists and companies started producing recombinant vaccines, hormones, and other drugs.
2002	The draft of human genome sequence was published.

Branches of biotechnology:

1-**Bioinformatics** is an recent field which addresses to resolve the biological problems using computational techniques, and makes the rapid organization as well as analysis of biological data possible. The field may be referred to **computational biology**, bioinformatics plays a key role in various areas, such as functional genomics, structural genomics, and forms a key component in the biotechnology and pharmaceutical.

2-Blue biotechnology is a term that has been used to describe the marine and aquatic applications of biotechnology.

3-Green biotechnology is biotechnology applied to agricultural processes. An example would be the selection and domestication of plants via micropropagation, transgenic plants. One hope is that green biotechnology might produce more environmentally friendly solutions than traditional industrial agriculture. An example of this is the engineering of a plant to express a pesticide.

4-Red biotechnology is applied to medical processes. Some examples are the designing of organisms to produce antibiotics, and the genetic manipulation.

5-White biotechnology is biotechnology applied to industrial processes. An example is the designing of an organism to produce a useful chemical, and using of enzymes as industrial catalysts to destroy polluting chemicals.

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Lec.( )

General applications of biotechnology:

Biotechnology is such an any branch of science which has advanced rapidly and providing benefits in all the fields of human.

A. Biotechnology in agriculture

For about 10,000 years, farmers have been improving wild plants through the selection and breeding for some characteristics. In the twentieth century, breeding became more complex, as the traits of breeders select for include increased yield, disease and pest resistance and drought resistance etc. Traits are passed from one generation to the next through genes, which are made of DNA.

Agricultural biotechnology is a collection of scientific techniques used to improve plants, based on an understanding of DNA, with ability to transfer specific gene to certain crops, This crops which have modified genomic called genetically modified crops (GM crops or biotech crops) and the process called genetic engineering techniques.

The transgenic plants may provide one or more characteristics of the following:

1- Resistance to insects, fungi, bacteria and virus
2- Highly resistant to herbicides, pesticides and other chemicals.
3- Drought, resistance, flood resistance, salinity resistance
4- High productivity and improved quality.

B. Biotechnology in medicines and health care:

In medicine, modern biotechnology finds applications in areas such as pharmaceutical drug discovery and production and genetic testing (or genetic screening).

The first genetically engineered products were medicines designed to treat human diseases. One example, in 1978 Genentech developed synthetic insulin by joining its gene with a plasmid vector inserted into the bacterium *Escherichia coli*. Insulin, widely used for the treatment of diabetes.

1- DNA monoclonal antibodies are used as tools for diagnosis of diseases.
2- Many value drugs and antibiotics are also produced on large scale by using biotechnological processes.
3- Human insulin was the first therapeutic product to be made commercially by genetically engineered bacterium.
4- **Gene therapy** is the method of curing genetic diseases (or acquired diseases) by the replacement of an abnormal gene by a therapeutic gene.

C. Industrial biotechnology

Is the application of biotechnology for industrial purposes, including industrial fermentation, and includes the using cells such as micro-organisms, or components of cells like enzymes, to generate industrially useful products such as chemicals, food and feed, detergents, paper and biofuels.

D. Biotechnology in energy and fuels

1. The microbes involved in fermentation can also be engineered for conversion of substrate into biofuel.
2. Biotechnology is contributing to increase the acceptability of biomass, biogas. etc.

3. There are certain plants which produce hydrocarbons and are called as petro-plants. e.g. rubber plants and certain algae.

E. Biotechnology and environment

1. Biological methods using organisms to breakdown the pollutants
2. Genetically engineered microbes are used for efficient treatment of industrial waste water.
3. A greatly enhanced oil-eating bacterial strain i.e. *Pseudomonas*.
4. Bioremediation of pollutants is an effective method of removal the earth's pollution. **Bioremediation** means the utilization of biological organisms for reducing pollution or for the removal of environmental pollutants. The bioremediation of organic toxic pollutants is mainly based on the microorganisms and thus it is called as 'microbial bioremediation. On the other hand, the bioremediation of inorganic contaminants is carried by certain plant species and therefore it is termed as **phytoremediation.**

Gene Therapy:

Gene therapy in most simple words is the **use of a gene to cure a disease**. There are a number of genetic diseases or acquired disorders that may have occurred due to specific mutations in genes. **They may be corrected by replacing the defective gene by a normal healthy gene, this strategy of correcting the diseases is termed as gene therapy.** So, the gene therapy may be **defined as the introduction of normal functional gene in the defective cells of a patient**. During 1940s it was discovered that a gene from one bacterial strain could be transferred into

another strain and also that gene could be expressed in another strain successfully. This discovery made the researchers to think about the possibility that human genetic disorder can be corrected in an analogous manner.

Types of gene therapy:

There are mainly two types of gene therapies:

(a) Somatic gene therapy:

In this type, the therapeutic gene is introduced in the somatic cells of the patient. The effect so produced is not heritable. Such as cancer and blood disorders.

(b) Germ line gene therapy:

In this type of gene therapy, the functional normal genes are introduced into the germ cells like sperm and eggs to correct the disorder. The changes produced by such system are heritable and thus are passed to the next generations

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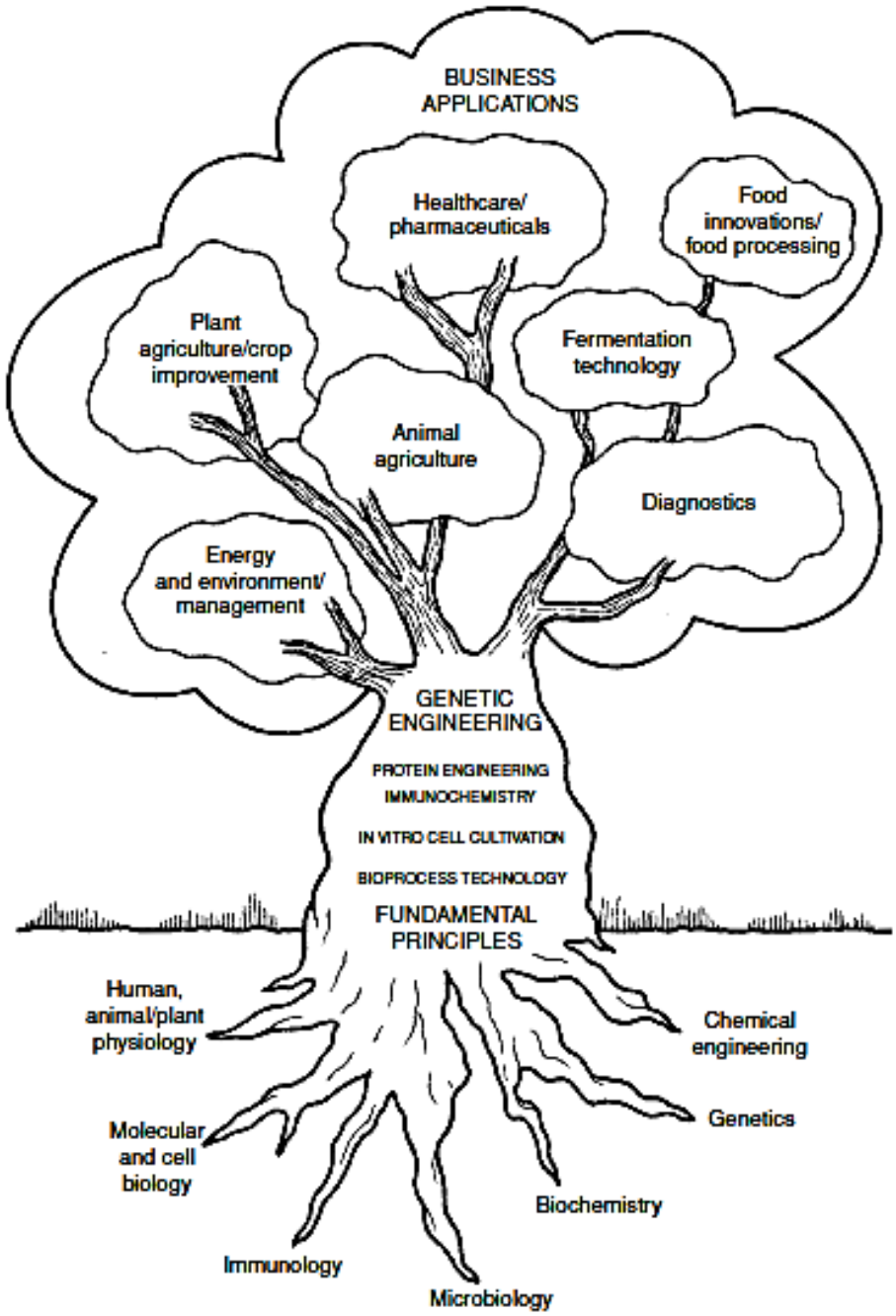

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Dr. Amjad A. Mohammed **Third class / Biology**

Animal Tissue Culture **Lec. ( ) / /2021**

Introduction

One of the most features of animals is that they are multicellular−in other words− they are composed of many cells. With this multicellularity comes the specialization of cells. In a multicellular organism, each cell does not have to carry out all the activities necessary for the life of the organism. Each cell type has its own role−to secrete a specific product, to contract, to transmit an electrical impulse, and so on. The result of this cellular specialization is that animals consist of a number of different types of cells – each with a characteristic size, shape, structure and function. A vertebrate has more than 100 different types of cells.

Animal tissue culture: is the growth of tissues separate from the animal *in vitro* (in the laboratory culture media).

Culture media of animal tissue culture

1- **Physical media**: such as matrix
2- **Chemical define media**: nutrients, hormones and stromal factors
3- **Natural media** (natural tissues extracts)
4- **Serum media** is the most economical, available and widely used culture medium for animal cell culture. Due to its **functions:** provide nutrients, hormones, growth factors, attachment factors, binding proteins, vitamins, minerals, lipids, protease inhibitors and pH buffer.

Disadvantages of serum:

- Virus, fungi and bacteria may contaminate the serum easily
- Some enzymes presents in serum can convert the cell secretions into toxic compounds.

Types of animal cell cultures:

A- Primary cell culture

The maintenance of growth of cells separated from the parental tissue in culture medium using suitable glass or plastic containers is called primary cell culture.

Disadvantages:

1. The preparation of primary cultures is labor intensive.
2. Can be maintained *in vitro* only for a limited period of time with limited number of cell divisions.
3. Very exposure to contamination.

There are two types of it:

1- **Adhesion cells**: Cells shown to require attachment for growth. They are usually derived from tissues of organs such as kidney.

2- **Suspension Culture**: Cells which do not require attachment for growth. They are derived from cells of the blood system.

B- Continuous Cultures (Secondary cell culture)

Derived from subculture of primary culture

There are two types of continuous cultures

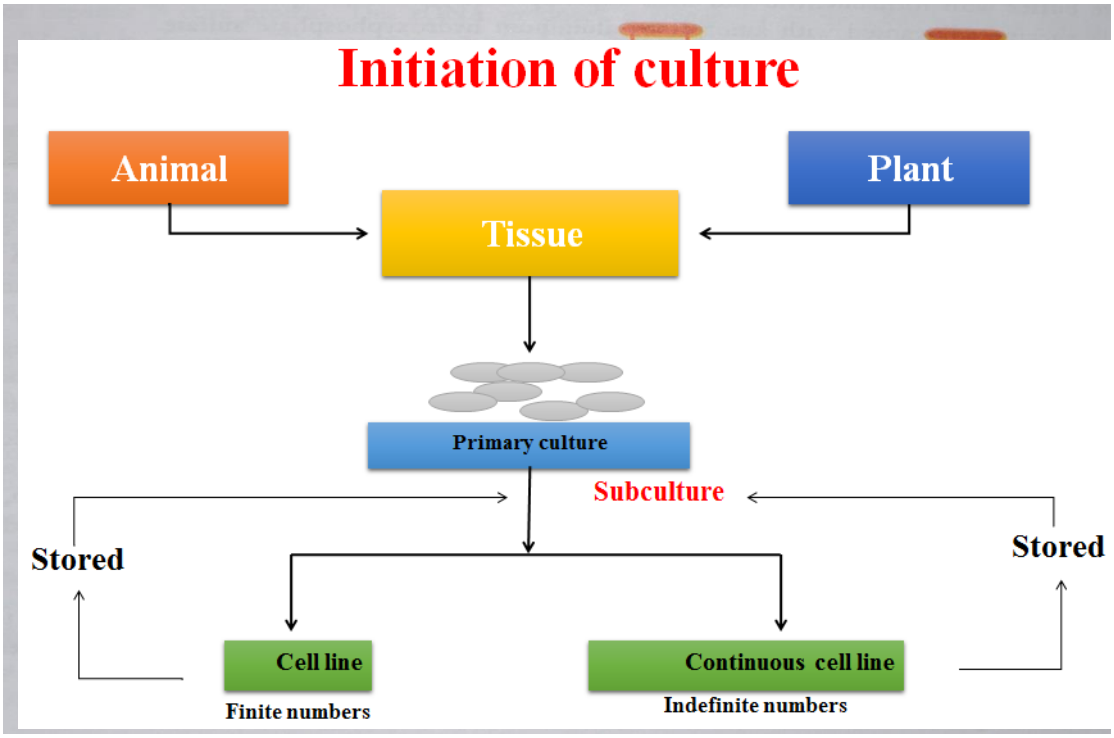
- Cell lines
- Continuous cell lines

Cell lines

1. Cell lines derived from primary cultures have a limited life span.
2. After the first subculture, the primary culture becomes cell line.
3. Senesce after approximately thirty cycles of division.

2) Continuous cell lines

1. can be propagated for long period because they have been transformed by:
   - tumor cells.
   - viral oncogenes
   - chemical treatments



Procedure for production of a cell line:

1- A piece of tissue is removed from an organism.

2- Adhesion between cells is broken with enzymes like trypsin or collagenase.

3- The cells are transferred to a plastic dish or bottle which contains culture medium.

4- The cells are incubated at control condition

5- The cells grow, divide and cover the surface of the container, this culture is referred to as **primary cell culture**.

6- The cells are transferred to a fresh medium and will again start growing.

7-After subculture obtained cell line

Applications of animal cell culture:

- ✓ To study the pattern of viral infection.
- ✓ They are used in the manufacture of vaccines, antibodies, hormones, interferon, vitamins, steroids, pharmaceutical drugs…etc.

- ✓ They are good tools for testing the potency of drugs.
- ✓ They are used in study of the effects of toxins and contaminants.
- ✓ Cancer research.

History of Animal Cell Culture:

1885: Roux maintained embryonic chick cells in a saline culture.

1907: Harrison cultivated frog nerve cells in a lymph clot held by the 'hanging drop' method and observed the growth of nerve fibres in vitro for several weeks. He was considered by some as the father of cell culture.

1910: Burrows succeeded in long-term cultivation of chicken embryo cell in plasma clots. He made detailed observation of mitosis.

1911: Lewis and Lewis made the first liquid media consisted of sea water, serum, embryo extract, salts and peptones. They observed limited monolayer growth.

1940s: The use of the antibiotics penicillin and streptomycin in culture medium decreased the problem of contamination in cell culture.

1948: Earle isolated mouse L fibroblasts which formed clones from single cells. Fischer developed a chemically defined medium, CMRL 1066.

1952: Gey established a continuous cell line from a human cervical carcinoma known as HeLa (Helen Lane) cells. Dulbecco developed plaque assay for animal viruses using confluent monolayers of cultured cells.

1954: Abercrombie observed contract inhibition: motility of diploid cells in monolayer culture ceases when contact is made with adjacent cells.

1955: Eagle studied the nutrient requirements of selected cells in culture and established the first widely used chemically defined medium.

1985: Human growth hormones produced from recombinant bacteria was accepted for therapeutic use.

1989: Recombinant erythropoietin in trial.

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General characteristics of anti-microbial drugs **Lec.( ) / / 2021**

— Anti-microbial drugs

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Antibiotic are:

- ✓ **Technically:** a substance produced by microorganism to inhibit or kill other microorganisms, e.g. the *Penicillium spp.* produces penicillin which kills Gram^{+} bacteria.
- ✓ **Practice**, is refer to any substance, natural or synthetic, that inhibits or kills microorganisms.
- ✓ **Therapeutically**: **antibiotics** are antimicrobial drugs.

Chemotherapy: The use of chemical drugs to treat a disease.

Spectrum of (activity) action of antibiotics: is the rang of effect the specific antibiotic on one pathogens target or more. There are two types:

- ✓ **Broad-spectrum antibiotics**: refers to drugs effective against more than one pathogens, exp. Tetracycline and Erythromycin.

- ✓ **Narrow-spectrum antibiotics**: refers to drugs effective against only one pathogens, exp. Isoniazid and Polymyxin.

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General characteristics of anti-microbial drugs **Lec.( ) / / 2021**

Features of antimicrobial drugs:

1. **Selective toxicity**: Drug kills pathogens without damaging the host.
2. **Therapeutic index**: ratio between toxic dose and therapeutic dose
3. **Antimicrobial action**: either **bacteriostatic** (inhibit growth of microorganisms) or **bactericidal** (kill microorganisms)

Different types of antimicrobial drugs:

1. Anti-bacterial drugs
2. Anti-fungal drugs
3. Anti-viral drugs
4. Anti-parasite drugs

Mechanisms the action of antibacterial drugs

1. Inhibit cell wall synthesis
2. Inhibit protein synthesis
3. Inhibit nucleic acid synthesis
4. Injury to plasma membrane
5. Inhibit synthesis of essential metabolites

Antimicrobial drug resistance : How its resistance obtained?

Resistance to an antibiotic is generally obtained by the acquired of antibiotic resistance genes: e.g., transformation, conjugation, or mutation.

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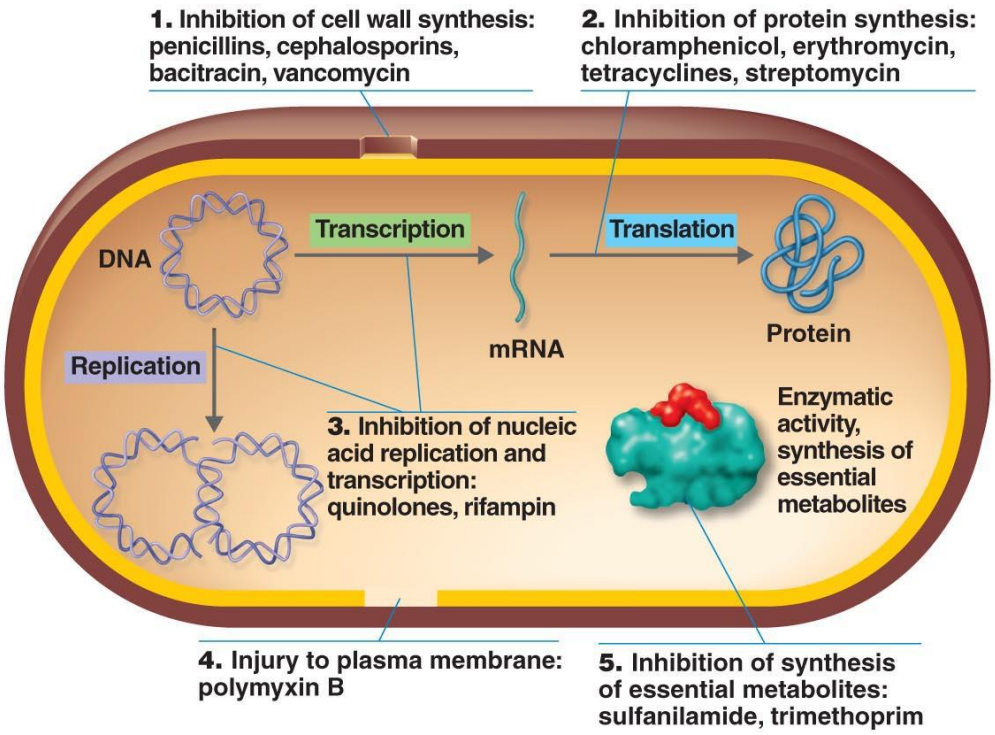
General characteristics of anti-microbial drugs **Lec.( ) / / 2021**



Resistance genes action can be of several types:

- Enzymes that degrade the antibiotic
- Changes in membrane proteins that limit entry of antibiotic
- molecular changes which prevent binding of the antibiotic to the target site.

Anti-mycobacterial drugs

Mycobacteria are difficult targets due to their outer membrane containing mycolic acids. The most effective antibiotics for mycobacterial infections are: Rifamycins, Isoniazid, Ethambutol.

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General characteristics of anti-microbial drugs **Lec.( ) / / 2021**

Mechanism of Action

Penicillin:

1. **Penicillin**: inhibits bacterial growth by interfering with the bacterial cell wall synthesis.
2. Penicillin are bactericidal drugs, the mechanisms by which they kill bacteria vary for different species.

Aminoglycosides:

1.**Aminoglycosides:** bind to ribosomes.
2. Are inhibition of protein synthesis and an error in reading the genetic code.

Tetracyclines:

-Tetracyclines: are **bacteriostatic** drugs and act on the bacterial ribosome.

-Inhibition of protein synthesis

Erythromycin:

An inhibitor of protein synthesis.

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General characteristics of anti-microbial drugs **Lec.( ) / / 2021**

General Principles of Antimicrobial Use:

A. Factors to be considered in the initial choice of antibiotics

1. The identity of the infecting organism must be known, or at the very least, it must be possible to make a probability assessment of the most likely culprit(s)
2. The likely antimicrobial susceptibility pattern of the invading organisms must be estimated.
3. Individual Hospital and ICU Variation

a. Particular issues today: Methicillin-resistant Staphylococcus aureus ("MRSA"); antibiotic resistant gram negative bacilli; Vancomycin, ampicillin, gentamycin-resistant enterococci ("VRE")

b. Possible issues tomorrow: penicillin-resistant pneumococci

4. The presence or absence of host factors that can modify the choice of antimicrobial agents

a. History of previous adverse reactions - must be specific as to nature of reaction. (e.g., nausea, vomiting, diarrhea not a major contraindication to repeat use of a drug; history of anaphylaxis or Stevens-Johnson syndrome is a major contraindication)

b. Age of patient:

- Neonates -chloramphenicol normally conjugated to glucuronide by liver; hepatic glucuronyl transferase levels in neonate very low, toxicity is very common sulfonamides compete with bilirubin for binding sites on serum albumin, can contribute to kernicterus
- Children -quinolones cause cartilage damage and arthropathy in young animals, therefore contraindicated in prepubescent children. tetracyclines - bind to developing bone and tooth structures, causing purplish brown discoloration of teeth, and even enamel hypoplasia.
- Elderly - isoniazid - above age 50, incidence of hepatotoxicity is 2.3%, under age 30 it is 0.3% increased nephrotoxicity with aminoglycosides and other similar drugs, likely secondary to decreased GFR associated with aging HST-151 13.

c. Pregnancy - As a general rule, the published data are totally inadequate for making recommendations. The following statements at present appear reasonable:

1) Penicillins (with the exception of ticarcillin), cephalosporins, and erythromycin are unlikely to be teratogenic and appear to be safe for use in pregnancy.
2) Metronidazole and ticarcillin are teratogenic in rodents and should never be used
3) Rifampin and trimethoprim should be avoided on theoretical grounds
4) Tetracyclines (in addition to effects on teeth of infant) are associated with fatty necrosis of the liver, pancreatitis, and probably renal damage in the pregnant woman
5) Aminoglycosides cross the placenta, ?effects on VIIIth nerve function of fetus. Ex: streptomycin
6) Isoniazid - ? associated with psychomotor retardation, myoclonus, and seizures in infant.

- Pharmacokinetics are altered in pregnancy - larger volume of distribution and more rapid clearance from blood, therefore lower serum levels.
- Essentially all antimicrobial agents appear in breast milk. Therefore, need to consider potential effects on infant

d. Genetic or metabolic abnormalities - This is an area that will expand rapidly in the next decade.

- Slow acetylators of INH (45-64% of Americans) - at risk for polyneuritis. Therefore give everyone pyridoxine.
- G6PD deficiency - sulfonamides, sulfones, nitrofurantoin, chloramphenicol will precipitate hemolysis

e. Renal and Hepatic function

- Dosage adjustment in renal dysfunction or failure is highly variable.
- As a general rule, the amount of dosage manipulation necessary in renal failure depends upon the extent to which nonrenal routes of clearance (primarily hepatobiliary) can compensate.

• Erythromycin, chloramphenicol, lincomycin, and clindamycin should be used with caution in patients with impaired hepatic function.

4. Site of Infection - the site of infection determines not only the choice of the agent but also its dose and the route by which it should be administered.

a. ability to achieve effective concentration at sites of interest: e.g., CSF

b. local factors that may modify drug efficacy

1. Pus

• Aminoglycosides and polymixins bind to (and are inactivated by) pus.

• Beta-lactamases produced by such organisms as Bacteroides fragilis can cause local inactivation of beta-lactam antibiotics at the site of mixed infection.

c. pH – e.g. aminoglycosides have low activity at low pH.

d. presence of foreign body.

B. Rational use of antimicrobial combinations in infectious disease process

1. For preventing emergence of resistant organisms

2. High probability of a polymicrobial infection

3. Provision of broad antimicrobial spectrum as initial therapy when patient seriously ill and etiology unclear.

4. Combination therapy to permit lower doses and decrease toxicity.

5. To achieve antimicrobial synergy

• only examples to be clinically proven to be of importance: penicillin + aminoglycoside for serious enterococcal infection; anti-pseudomonal beta-lactam + tobramycin for Pseudomonas amphotericin + flucytosine for *Cryptococcus* neoformans

• many examples of test tube synergy with questionable clinical importance.

6. Disadvantages of antimicrobial combinations:

• Antagonism

• Cost

• Side-effects

C. Choice of route of administration.

In addition to issues related to the intrinsic pharmacokinetic properties of a drug, the major reasons for utilizing parenteral therapy (usually IV) are:

1. Serious illness that requires immediately achieving high blood and tissue concentrations
2. Inadequate GI tract function - i.e., the presence of ileus, nausea and vomiting, etc.

D. First two commandments of antimicrobial therapy

1. Buy Time. The first concern is to keep the patient alive until you know the etiology and antimicrobial susceptibility of the invading pathogen and thereby precisely target treatment. Up until that point, you need to make this important distinction:

a) therapeutic emergency -- "front load" antibiotics

b) diagnostic dilemma -- "after load" antibiotics.

2. Look for abnormality. The second question is whether the patient has an abnormality that increases the risk from an inadequately treated bacteremia. Ex: abnormal heart valve, prosthetic joint, prosthetic vascular graft. If yes, "front load" with bactericidal therapy.

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Genetic engineering **Lec. ( ) / /2021**

Genetic engineering is the artificial manipulation and modification in nucleic acid molecules to modify an organisms and recombination of DNA production.

The term genetic engineering initially referred to various techniques used for the modification or manipulation of organisms through the processes of heredity and reproduction. As such, the term include both artificial selection and all the interventions of biomedical techniques, among them artificial inoculations, *in vitro* fertilization (e.g., "test-tube" babies), cloning, and gene manipulation. In the latter part of the 20th century, however, the term came to refer more specifically to methods of recombinant DNA technology (or gene cloning), in which DNA molecules from two or more sources are combined either within cells or *in vitro* and are then inserted into host organisms in which they are able to propagate.

History of GMO Development

- **1973: created first genetically modified bacteria**
- 1974: created GM mice
- 1982: first commercial development of GMOs (insulin-producing bacteria)
- 1994: began to sell genetically modified food
- 2003: began to sell GMOs as pets (Glofish)

Genetic modification can be completed by a number of different methods:

- ✓ Inserting new genetic material randomly or in targeted locations
- ✓ Direct replacement of genes (recombination)
- ✓ Removal of genes
- ✓ Mutation of existing genes

5 Stages involved in Genetic engineering (gene cloning)

1. Isolation
2. Cutting
3. Ligation and Insertion
4. Transformation
5. Expression

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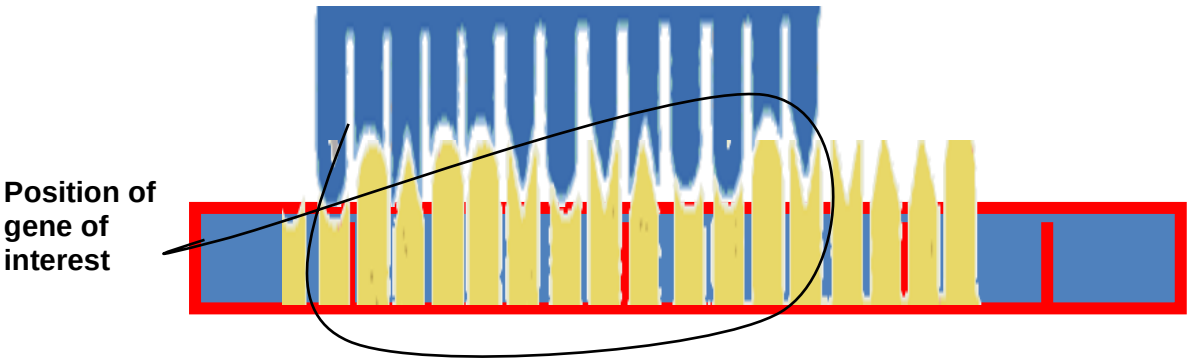
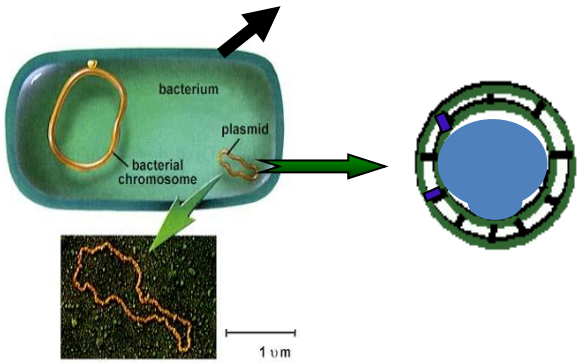
1-Isolation

(a) Isolation of a specific gene from donor e.g. human

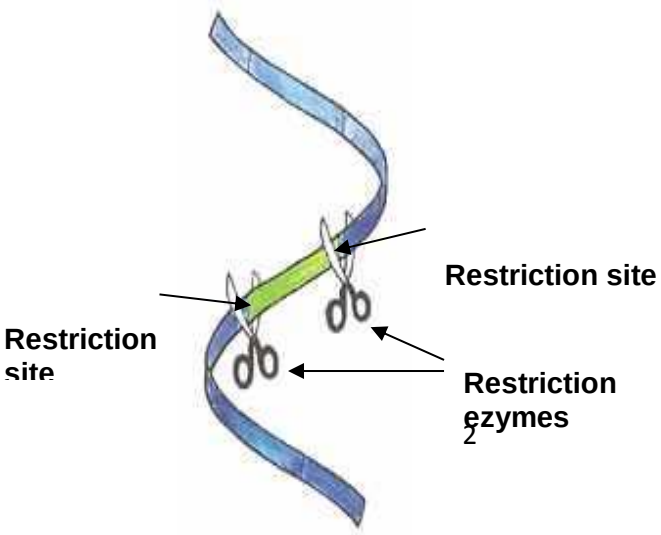


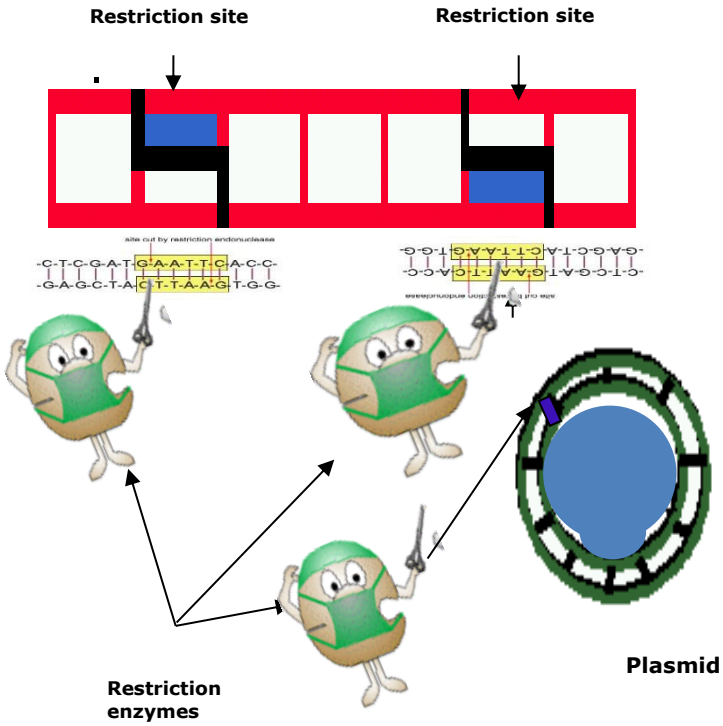
(b) Isolation of plasmid from a bacterial cell



2-Cutting

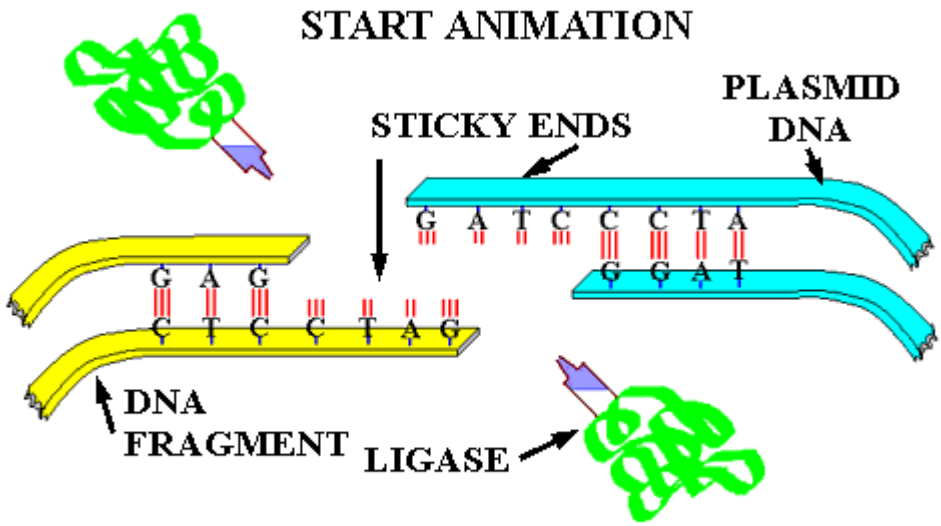
Restriction enzymes act as molecular scissors and cut DNA at specific sites called restriction sites

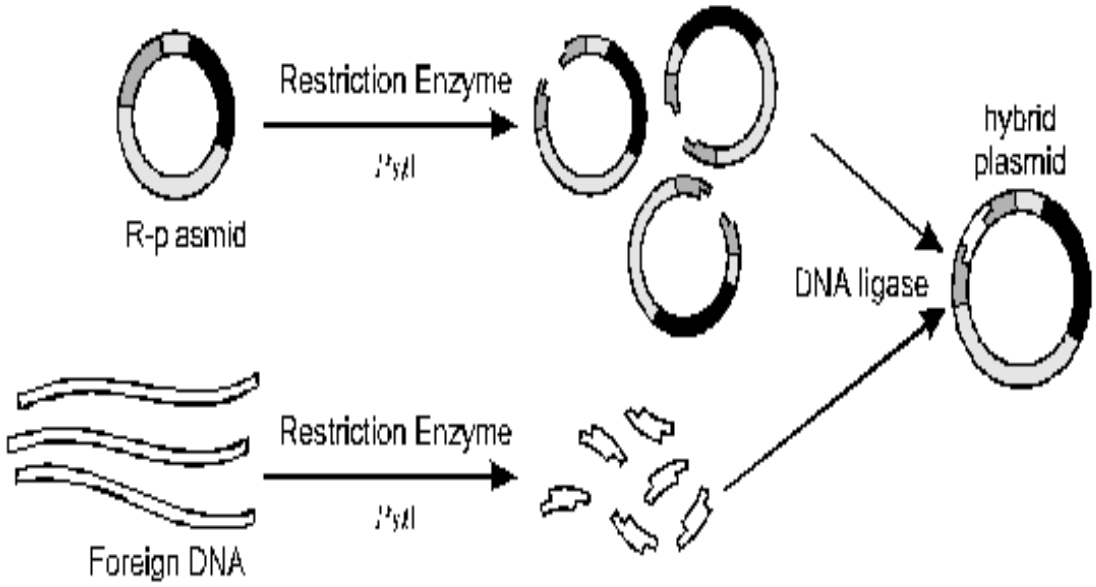




3-Ligation and Insertion

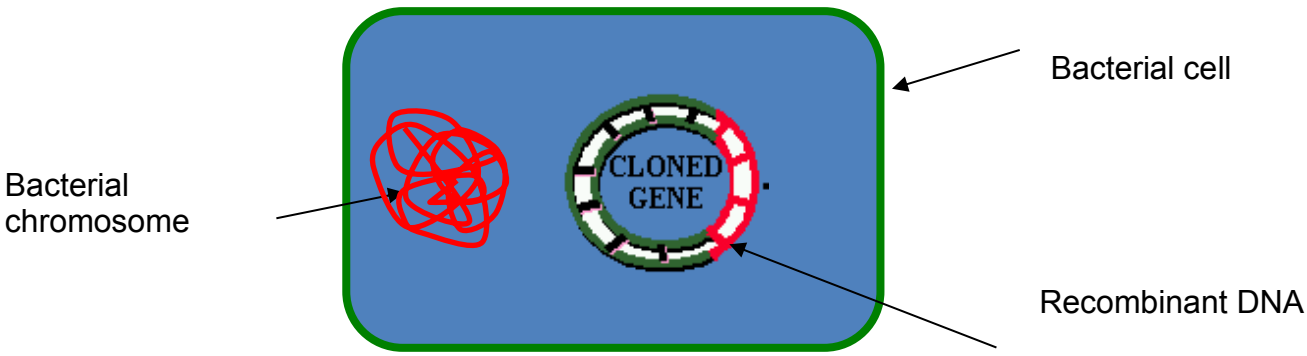
Ligation: re-joining cut fragments of DNA and forming artificial recombinant molecules





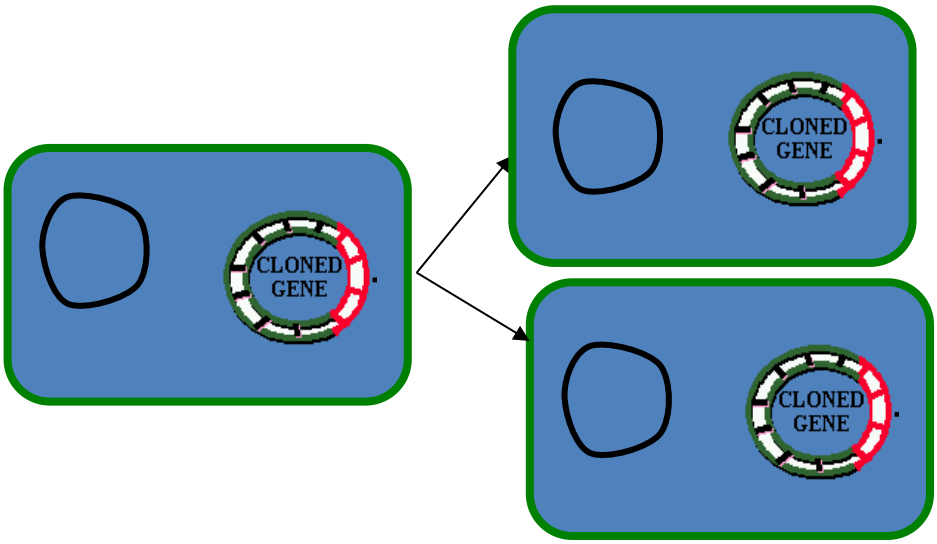
4-**Transformation**

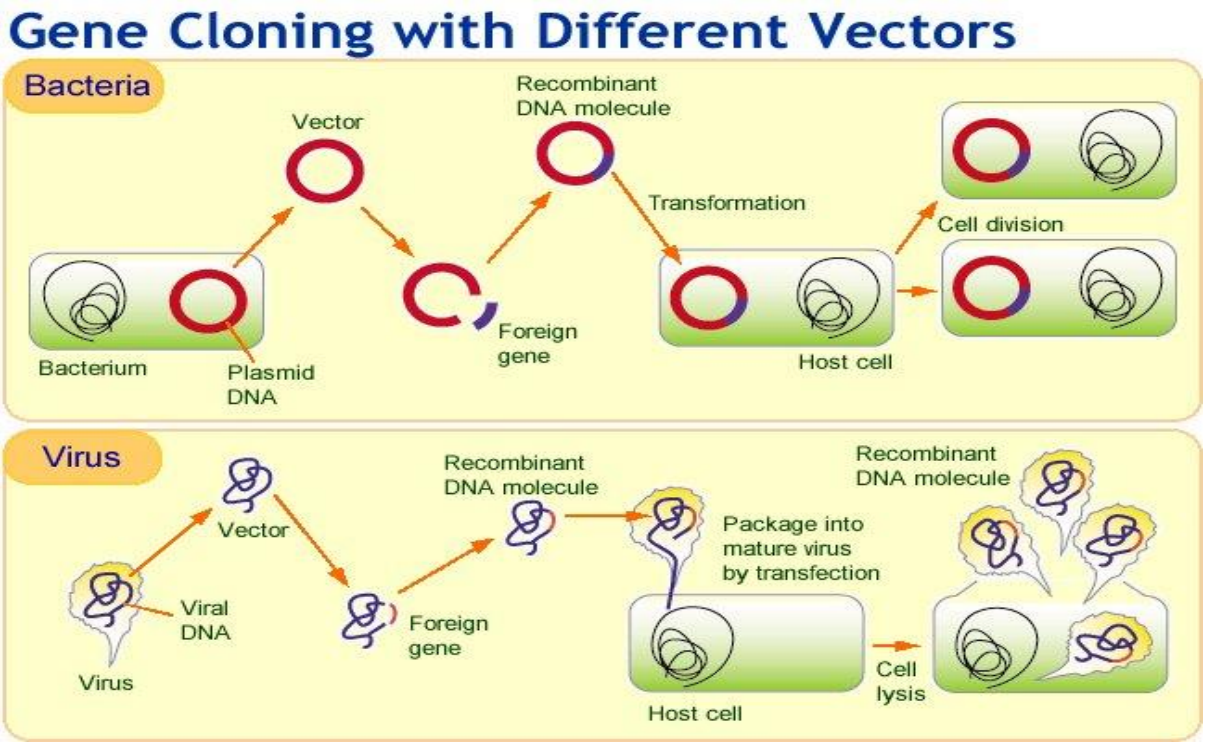
Recombinant DNA introduced into bacterial cell



5- Expression

- Bacterial cell reproduces by Binary Fission
- Bacterial cell produces the polypeptide
- Coded for by the donor DNA





BIOTECHNOLOGY

Dr. Amjad A. Mohammed **Third class / Biology**

Plant Transformation in Biotechnology **Lec. ( ) / /2021**

Plant Transformation in Biotechnology

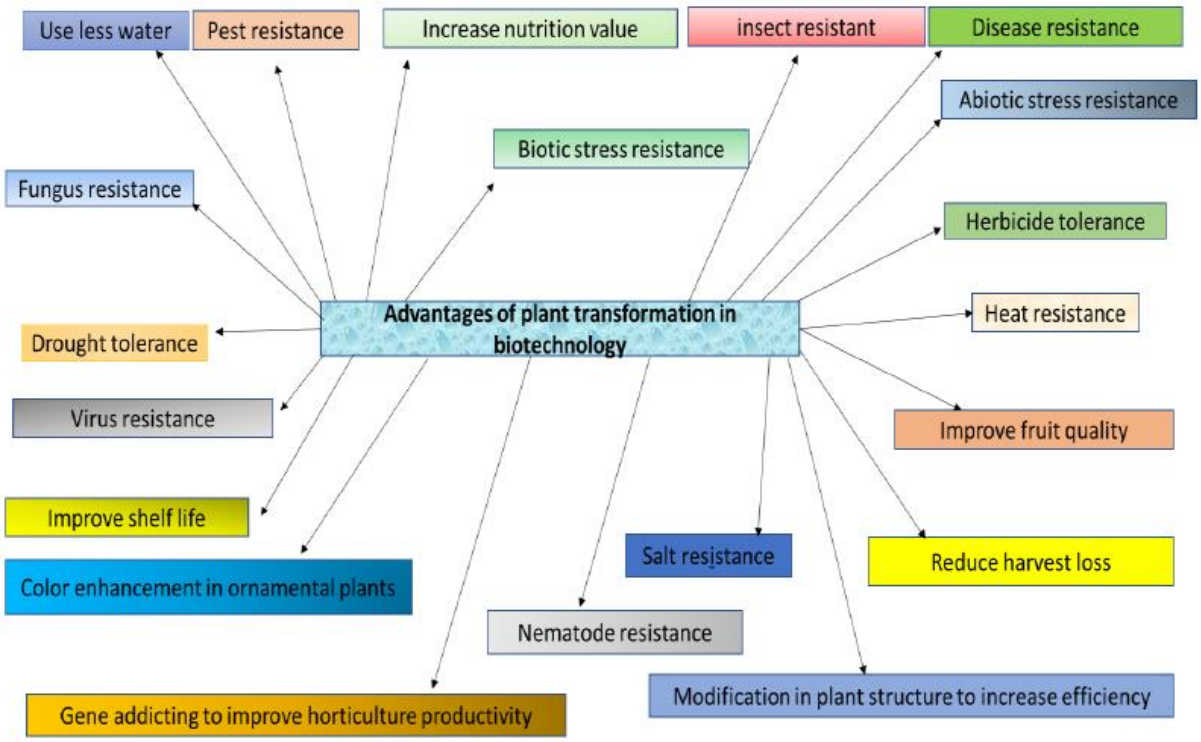
Plant transformation **permits the introduction of the gene of interest for producing a novel transgenic plants**. "When a gene from one species is moved or relocated to another species by using recombinant DNA technology are called **genetically modified organisms**. Genetic engineering is one way to modify the plants by selecting for desired traits. Genetically modified organisms have foreign genes derive from not only plant source but also from bacteria, viruses, fungi, insects and animals.

Transformation states to the introduction of a foreign gene of choice into the genome of the plant to the generation of transgenic plant. **Plant transformation is the incorporation of the foreign DNA into a plant genome (nuclear or cytoplasmic)**.

Plant transformation involves three phases:

- ✓ Target gene
- ✓ Plant tissues
- ✓ Vector for successful transformation.

Advantages of plant transformation



Methods of plant transformation

There are many methods of a direct and indirect way of plant transformation.

Physical methods of plant transformation

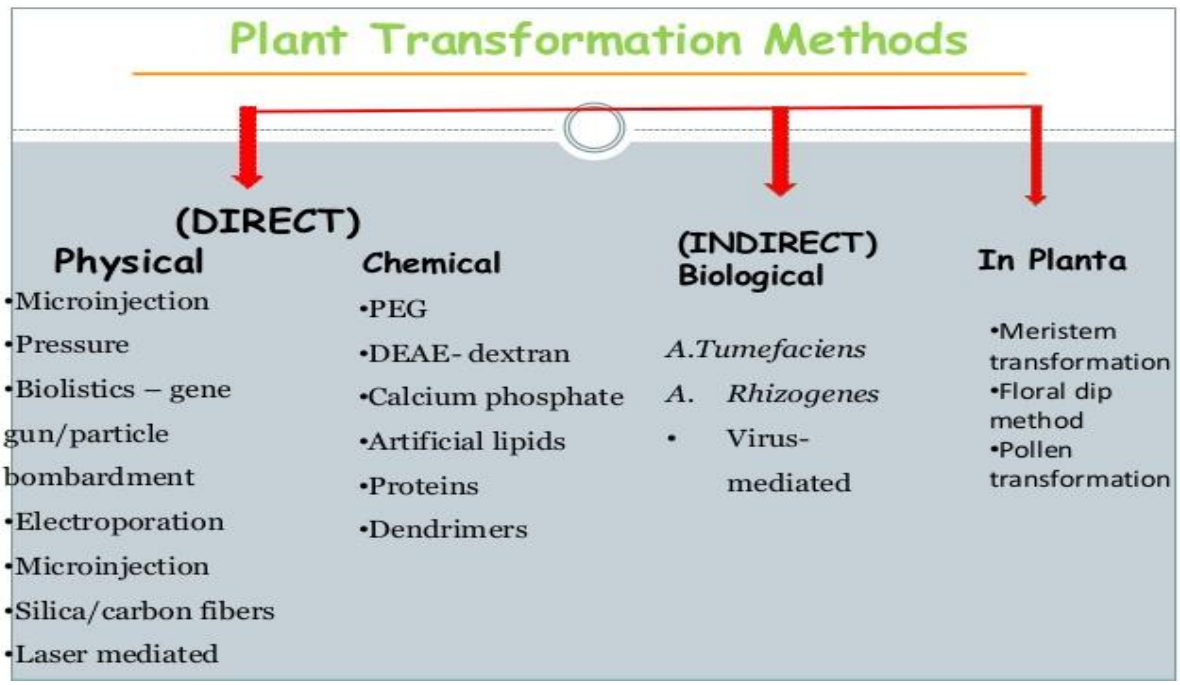
Genetic engineering is used to change the sequence of the genome of plant species. Modification in the construction and composition of genetic material in a living organism. There are two methods for genetic transformation indirect or direct transformation:

1-Direct methods

Direct methods are micro-injection, biolistic or gene gun methods, electroporation, silica carbide, microinjection, lipofection, sonification, calcium phosphate method, transfer of DNA by use of polyethylene glycol.

2-Indirect methods are *Agrobacterium*-mediated transfer, rhizobium mediated transfer.

The most published techniques for gene transfer into plant cells are DNA transfer by artificial methods like DNA transfer in physical methods are micro-injection, biolistic or gene gun methods, electroporation, silica carbide, microinjection, lipofection, microinjection. DNA also transfer by chemicals methods. In natural method like in biological method, *Agrobacterium*-mediated transfer, *Rhizobium*, virus-mediated



DNA transfer by artificial method by physical method

Microinjection

The microinjection is the procedure in which transporting foreign DNA into the cell through the microneedle and direct DNA inject in the nucleus under microscope.

Protoplast electroporation method

In this process, high electric shocks use to generate small holes in the cell membrane of the plant which allow the DNA to enter from these holes. These holes create for a short time less than 5 seconds. This process needs protoplast formation before transfer DNA because large molecule can't cross the cell membrane of the plant. Then after passing the pores of cell membrane DNA become the part of the chromosome of the plant nucleus.

Chemical methods

There are two methods of chemicals DNA transformation:

1-DNA transfer by calcium phosphate

In this method of gene transformation, a proper solution of DNA and of calcium chloride (CaCl) and phosphate(P) is made. Then DNA becomes precipitation with the calcium phosphate. Then precipitation of DNA transfer to the target tissue or cell.

2-Transfer of DNA by use of polyethylene glycol (PEG)

The first step of gene transfer by polyethylene glycol (PEG) is protoplast preparation in which the removal of cell wall through enzymatic digestion occurs. The protoplast of desire plant treated with polyethylene glycol (PEG) with the solution of DNA. Protoplasts are beneficial fragments for genetic engineering for the reason that they are more totipotent, which have quick ability to grow. In this technique Protoplasts of plants are cultivated in the presence of optimized conditions that are suitable for growing daughter plant with the desired character with the help of callus, shoots, and roots, and redevelop into whole plants PEG induces reversible permeabilization of the plasma membrane.

DNA transfer through natural or biological methods

There are many methods to transfer gene through natural methods:

1-CLONING

2-AGROBACTERIUM-mediated

Agrobacterium is a direct technique that is best known as one of natural agents in producing plant genetically modified organisms (GMOs). It attacks only dicotyledonous plants (and not easy on monocots plants). **The *Agrobacterium* divided to the two types**:

A. ***tumefaciens* cause crown gall disease** according to its plasmid Ti (tumor-inducing) plasmid
B. **A. *rhizogenes*** cause hairy roots diseases according to the presence Ri (Root- inducing) plasmid

So, *Agrobacterium* effects only injured plant because the injured plant produces phenolic compounds like acetosyringone. So, it activates the making of the copy of T-DNA in bacteria. Scientist study crown gall tumor of the plant than they found that tumor cell has not any tumor causing hormone to grow callus (auxin and cytokine) and it have same genes as *Agrobacterium* .So they found the reason that tumor caused by *Agrobacterium*. So, the tumor cell has both chromosomal DNA and also plasmid DNA of *Agrobacterium* bacteria. (*Agrobacterium tumefaciens*) or hairy root (*A. rhizogenes*) that are able to produce long hairy roots.

A minor portion of this plasmid, the T-DNA is transferred from the bacteria to the plant and participates steadily into the plant genome. By removing disease cause genes, they it become a vector. Mostly *A. tumefaciens* and the Ti-plasmid used because due to more mutual and large knowledge. Scientist reported that *A. tumefaciens* have its plasmid, which contains a series of different eleven vir genes (virG, virE, virA,

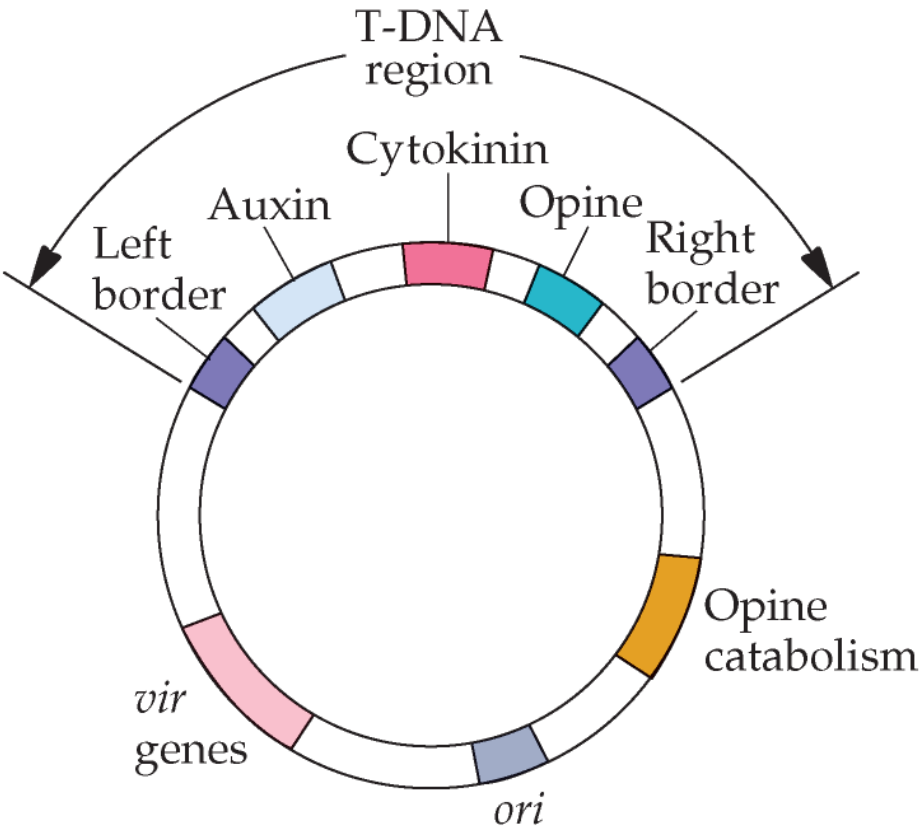
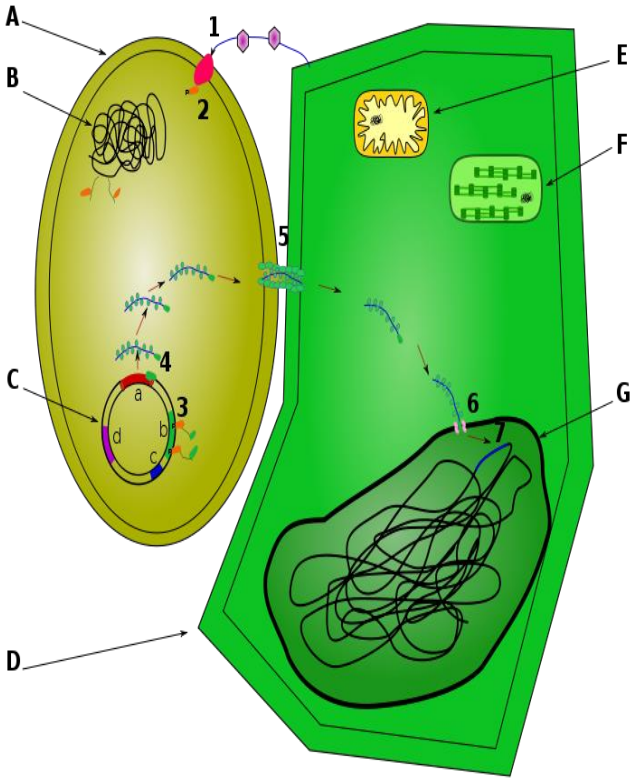


Figure: Ti plasmid structure and function. Note the wound-induced plant phenolics induce the *vir* genes on the Ti plasmid.

steps of infection:



A: *Agrobacterium tumefaciens*

B: Agrobacterium genome

C: Ti Plasmid :
- **a: T-DNA**
- **b: *Vir* genes**
- **c: Replication origin**
- **d: Opines catabolism genes**

D: Plant cell

E: Mitochondria

F: Chloroplast

G: Nucleus

Steps 1 and 2: Bacterial cell weakly attaches itself to the plant cell. It then produces cellulose fibrils to anchor it to the plant cell (infection).
Step 3: When the bacterium detects certain compounds produced by the plant in response to bacterial infection, *vir* (virulence) genes [located on b of the Ti plasmid (C)] start producing various compounds.
Step 4: One *vir* gene complex cuts the T-DNA (a) from the Ti plasmid (C).
Step 5: In the meantime, other *vir* genes produce compounds that coat the T-DNA to help export it into the recipient plant cell

Step 6: Other *vir* genes make the nucleus of the plant cell receiving the T-DNA more receptive.

Step 7: T-DNA is integrated into the host genome.

T-DNA contains genes that will force the plant to produce special amino acids called **opines**, which the bacteria can metabolize as its food source.