SRINIVASAN COLLEGE OF ARTS & SCIENCE

(Affiliated to Bharathidasan University, Trichy)

PERAMBALUR – 621 212.

DEPARTMENT OF MICROBIOLOGY

Course : B.Sc Year: **III** Semester: **VI**

Course Material on:

MICROBIAL BIOTECHNLOGY & BIOETHICS

Sub. Code : 16SMBEMB3

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Month & Year : APRIL – 2020

MICROBIAL BIOTECHNOLOGY AND BIOETHICS

UNIT I:

Biotechnology – Definition:

Biotechnology, the use of biology to solve problems and make useful products. The most prominent area of *biotechnology* is the production of therapeutic proteins and other drugs through genetic engineering.

History of Biotech can be divided into three phases:

1. Ancient Biotechnology
2. Classical Biotechnology
3. Modern Biotechnology

Ancient Biotechnology (Pre-1800)

Most of the biotech developments before the year 1800 can be termed as 'discoveries' or 'developments'. If we study all these developments, we can conclude that these inventions were based on common observations about nature.

- Humans have used biotechnology since the dawn of civilization.
- After domestication of food crops (corn, wheat) and wild animals, man moved on to other new observations like cheese and curd. Cheese can be considered as one of the first direct products (or by-product) of biotechnology because it was prepared by adding rennet (an enzyme found in the stomach of calves) to sour milk.
- Yeast is one of the oldest microbes that have been exploited by humans for their benefit. The oldest fermentation was used to make beer in Sumeria and Babylonia as early as 7,000BCE.
- By 4,000BCE, Egyptians used yeasts to bake leavened bread.
- Another ancient product of fermentation was wine, made in Assyria as early as 3,500BCE.
- The Chinese developed fermentation techniques for brewing and cheese making.
- 500 BCE: In China, the first antibiotic, moldy soybean curds, is put to use to treat boils.
- Hippocrates treated patients with vinegar in 400 BCE.
- In 100BCE, Rome had over 250 bakeries which were making leavened bread.
- A.D. 100: The first insecticide is produced in China from powdered chrysanthemums.
- The use of molds to saccharify rice in the koji process dates back to at least A.D. 700.
- 13th century: The Aztecs used Spirulina algae to make cakes.
- One of the oldest examples of crossbreeding for the benefit of humans is mule. Mule is an offspring of a male donkey and a female horse. People started using mules for transportation, carrying loads, and farming, when there were no tractors or trucks.

- By the 14th century AD, the distillation of alcoholic spirits was common in many parts of the world.
- Vinegar manufacture began in France at the end of the 14th century.
- 1663: Cells are first described by Hooke.
- 1673-1723: In the seventeenth century, Antonie van Leeuwenhoek discovered microorganisms by examining scrapings from his teeth under a microscope.
- 1675: Leeuwenhoek discovers protozoa and bacteria.
- 1761: English surgeon Edward Jenner pioneers vaccination, inoculating a child with a viral smallpox vaccine.

Classical Biotechnology (1800-1945)

The Hungarian Károly Ereky coined the word "biotechnology" in Hungary during 1919 to describe a technology based on converting raw materials into a more useful product. In a book entitled Biotechnologie, Ereky further developed a theme that would be reiterated through the 20th century: biotechnology could provide solutions to societal crises, such as food and energy shortages.

- 1773-1858: Robert Brown discovered the nucleus in cells.
- 1802: The word "biology" first appears.
- 1822-1895: Vaccination against small pox and rabies developed by Edward Jenner and Louis Pasteur.
- In 1850, Casimir Davaine detected rod-shaped objects in the blood of anthrax-infected sheep and was able to produce the disease in healthy sheep by inoculation of such blood.
- 1855: The Escherichia coli bacterium is discovered. It later becomes a major research, development, and production tool for biotechnology.
- In 1868, Fredrich Miescher reported nuclein, a compound that consisted of nucleic acid that he extracted from white blood cells.
- 1870: Breeders crossbreed cotton, developing hundreds of varieties with superior qualities.
- 1870: The first experimental corn hybrid is produced in a laboratory.
- By 1875, Pasteur of France and John Tyndall of Britain finally demolished the concept of spontaneous generation and proved that existing microbial life came from preexisting life.
- 1876: Koch's work led to the acceptance of the idea that specific diseases were caused by specific organisms, each of which had a specific form and function.
- In 1881, Robert Koch, a German physician, described bacterial colonies growing on potato slices (First ever solid medium).
- In 1888, Heinrich Wilhelm Gottfried Von Waldeyer-Hartz, a German scientist, coined the term 'Chromosome.'
- In 1909, the term 'Gene' had already been coined by Wilhelm Johannsen (1857-1927), who described 'gene' as carrier of heredity. Johannsen also coined the terms 'genotype' and 'phenotype.'
- 1909: Genes are linked with hereditary disorders.

- 1911: American pathologist Peyton Rous discovers the first cancer-causing virus.
- 1915: Phages, or bacterial viruses, are discovered.
- 1919: The word “biotechnology” is first used by a Hungarian agricultural engineer.
- Pfizer, which had made fortunes using fermenting processes to produce citric acid in the 1920s, turned its attention to penicillin. The massive production of penicillin was a major factor in the Allied victory in WWII.
- 1924: start of Eugenic Movement in the US.
- The principle of genetics in inheritance was redefined by T.H. Morgan, who showed inheritance and the role of chromosomes in inheritance by using fruit flies. This landmark work was named, ‘The theory of the Gene in 1926.”
- Alexander Fleming discovered ‘penicillin’ the antibacterial toxin from the mold Penicillium notatum, which could be used against many infectious diseases. Fleming wrote, “When I woke up just after dawn on September 28, 1928, I certainly didn’t plan to revolutionize all medicine by discovering the world’s first antibiotic, or bacteria killer.”
- 1933: Hybrid corn is commercialized.
- In 1940, a team of researchers at Oxford University found a way to purify penicillin and keep it stable.
- 1941: The term “genetic engineering” is first used by a Danish microbiologist.
- 1942: The electron microscope is used to identify and characterize a bacteriophage- a virus that infects bacteria.
- 1942: Penicillin is mass-produced in microbes for the first time.

Modern Biotechnology (1945-present)

The Second World War became a major impediment in scientific discoveries. After the end of the second world war some, very crucial discoveries were reported, which paved the path for modern biotechnology.

The origins of biotechnology culminate with the birth of genetic engineering. There were two key events that have come to be seen as scientific breakthroughs beginning the era that would unite genetics with biotechnology: One was the 1953 discovery of the structure of DNA, by Watson and Crick, and the other was the 1973 discovery by Cohen and Boyer of a recombinant DNA technique by which a section of DNA was cut from the plasmid of an E. coli bacterium and transferred into the DNA of another. Popularly referred to as “genetic engineering,” it came to be defined as the basis of new biotechnology.

In Britain, Chaim Weizemann (1874–1952) developed bacterial fermentation processes for producing organic chemicals such as acetone and cordite propellants. During WWII, he worked on synthetic rubber and high-octane gas.

- 1950s: The first synthetic antibiotic is created.
- 1951: Artificial insemination of livestock is accomplished using frozen semen.

- In 1953, JD Watson and FHC Crick for the first time cleared the mysteries around the DNA as a genetic material, by giving a structural model of DNA, popularly known as, 'Double Helix Model of DNA.'
- 1954: Dr. Joseph Murray performs the first kidney transplant between identical twins.
- 1955: An enzyme, DNA polymerase, involved in the synthesis of a nucleic acid, is isolated for the first time.
- 1955: Dr. Jonas Salk develops the first polio vaccine. The development marks the first use of mammalian cells (monkey kidney cells) and the first application of cell culture technology to generate a vaccine.
- 1957: Scientists prove that sickle-cell anemia occurs due to a change in a single amino acid in hemoglobin cells
- 1958: Dr. Arthur Kornberg of Washington University in St. Louis makes DNA in a test tube for the first time.
- Edward Tatum (1909–1975) and Joshua Lederberg (1925–2008) shared the 1958 Nobel Prize for showing that genes regulate the metabolism by producing specific enzymes.
- 1960: French scientists discover messenger RNA (mRNA).
- 1961: Scientists understand genetic code for the first time.
- 1962: Dr. Osamu Shimomura discovers the green fluorescent protein in the jellyfish Aequorea victoria. He later develops it into a tool for observing previously invisible cellular processes.
- 1963: Dr. Samuel Katz and Dr. John F. Enders develop the first vaccine for measles.
- 1964: The existence of reverse transcriptase is predicted.
- At a conference in 1964, Tatum laid out his vision of "new" biotechnology: "Biological engineering seems to fall naturally into three primary categories of means to modify organisms. These are: 1. The recombination of existing genes, or eugenics. 2. The production of new genes by a process of directed mutation, or genetic engineering. 3. Modification or control of gene expression, or to adopt Lederberg's suggested terminology, euphenic engineering."
- 1967: The first automatic protein sequencer is perfected.
- 1967: Dr. Maurice Hilleman develops the first American vaccine for mumps.
- 1969: An enzyme is synthesized in vitro for the first time.
- 1969: The first vaccine for rubella is developed.
- 1970: Restriction enzymes are discovered.
- 1971: The measles/mumps/rubella combo-vaccine was formed.
- 1972: DNA ligase, which links DNA fragments together, is used for the first time.
- 1973: Cohen and Boyer perform the first successful recombinant DNA experiment, using bacterial genes.

- In 1974, Stanley Cohen and Herbert Boyer developed a technique for splicing together strands of DNA from more than one organism. The product of this transformation is called recombinant DNA (rDNA).
- Kohler and Milestein in 1975 came up with the concept of cytoplasmic hybridization and produced the first ever monoclonal antibodies, which has revolutionized diagnostics.
- Techniques for producing monoclonal antibodies were developed in 1975.
- 1975: Colony hybridization and Southern blotting are developed for detecting specific DNA sequences.
- 1976: Molecular hybridization is used for the prenatal diagnosis of alpha thalassemia.
- 1978: Recombinant human insulin is produced for the first time.
- 1978: with the development of synthetic human insulin the biotechnology industry grew rapidly.
- 1979: Human growth hormone is synthesized for the first time.
- In the 1970s-80s, the path of biotechnology became intertwined with that of genetics.
- By the 1980s, biotechnology grew into a promising real industry.
- 1980: Smallpox is globally eradicated following 20-year mass vaccination effort.
- In 1980, The U.S. Supreme Court (SCOTUS), in Diamond v. Chakrabarty, approved the principle of patenting genetically engineered life forms.
- 1981: Scientists at Ohio University produce the first transgenic animals by transferring genes from other animals into mice.
- 1981: The first gene-synthesizing machines are developed.
- 1981: The first genetically engineered plant is reported.
- 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. This is followed by many new drugs based on biotechnologies.
- 1983: The discovery of HIV/AIDS as a deadly disease has helped tremendously to improve various tools employed by life-scientist for discoveries and applications in various aspects of day-to-day life.
- In 1983, Kary Mullis developed polymerase chain reaction (PCR), which allows a piece of DNA to be replicated over and over again. PCR, which uses heat and enzymes to make unlimited copies of genes and gene fragments, later becomes a major tool in biotech research and product development worldwide.
- 1983: The first artificial chromosome is synthesized.
- In 1983, the first genetic markers for specific inherited diseases were found.
- 1983: The first genetic transformation of plant cells by TI plasmids is performed.
- In 1984, the DNA fingerprinting technique was developed.

- 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.
- 1986: University of California, Berkeley, chemist Dr. Peter Schultz describes how to combine antibodies and enzymes (abzymes) to create therapeutics.
- 1988: The first pest-resistant corn, Bt corn, is produced.
- 1988: Congress funds the Human Genome Project, a massive effort to map and sequence the human genetic code as well as the genomes of other species.
- In 1988, chymosin (known as Rennin) was the first enzyme produced from a genetically modified source-yeast-to be approved for use in food.
- In 1988, only five proteins from genetically engineered cells had been approved as drugs by the United States Food and Drug Administration (FDA), but this number would skyrocket to over 125 by the end of the 1990s.
- In 1989, microorganisms were used to clean up the Exxon Valdez oil spill.
- 1990: The first successful gene therapy is performed on a 4-year-old girl suffering from an immune disorder.
- In 1993, The U.S. Food and Drug Administration (FDA) declared that genetically modified (GM) foods are "not inherently dangerous" and do not require special regulation.
- 1993: Chiron's Betaseron is approved as the first treatment for multiple sclerosis in 20 years.
- 1994: The first breast cancer gene is discovered.
- 1995: Gene therapy, immune-system modulation and recombinantly produced antibodies enter the clinic in the war against cancer.
- 1995: The first baboon-to-human bone marrow transplant is performed on an AIDS patient.
- 1995: The first vaccine for Hepatitis A is developed.
- 1996: A gene associated with Parkinson's disease is discovered.
- 1996: The first genetically engineered crop is commercialized.
- 1997: Ian Wilmut, an Irish scientist, was successful in cloning an adult animal, using sheep as model and naming the cloned sheep 'Dolly.'
- 1997: The first human artificial chromosome is created.
- 1998: A rough draft of the human genome map is produced, showing the locations of more than 30,000 genes.
- 1998: Human skin is produced for the first time in the lab.
- 1999: A diagnostic test allows quick identification of Bovine Spongicorm Encephalopathy (BSE, also known as "mad cow" disease) and Creutzfeldt-Jakob Disease (CJD).
- 1999: The complete genetic code of the human chromosome is deciphered.

- 2000: Kenya field-tests its first biotech crop, virus-resistant sweet potato.
- Craig Venter, in 2000, was able to sequence the human genome.
- 2001: The sequence of the human genome is published in Science and Nature, making it possible for researchers all over the world to begin developing treatments.
- 2001: FDA approves Gleevec® (imatinib), a gene-targeted drug for patients with chronic myeloid leukemia. Gleevec is the first gene-targeted drug to receive FDA approval.
- 2002: EPA approves the first transgenic rootworm-resistant corn.
- 2002: The banteng, an endangered species, is cloned for the first time.
- 2003: China grants the world's first regulatory approval of a gene therapy product, Gendicine (Shenzhen SiBiono GenTech), which delivers the p53 gene as a therapy for squamous cell head and neck cancer.
- In 2003, TK-1 (GloFish) went on sale in Taiwan, as the first genetically modified pet.
- 2003: The Human Genome Project completes sequencing of the human genome.
- 2004: UN Food and Agriculture Organization endorses biotech crops, stating biotechnology is a complementary tool to traditional farming methods that can help poor farmers and consumers in developing nations.
- 2004: FDA approves the first antiangiogenic drug for cancer, Avastin®.
- 2005: The Energy Policy Act is passed and signed into law, authorizing numerous incentives for bioethanol development.
- 2006: FDA approves the recombinant vaccine Gardasil®, the first vaccine developed against human papillomavirus (HPV), an infection implicated in cervical and throat cancers, and the first preventative cancer vaccine.
- 2006: USDA grantsDow AgroSciences the first regulatory approval for a plant-made vaccine.
- 2006: The National Institutes of Health begins a 10-year, 10,000-patient study using a genetic test that predicts breast-cancer recurrence and guides treatment.
- In 2006, the artist Stelarc had an ear grown in a vat and grafted onto his arm.
- 2007: FDA approves the H5N1 vaccine, the first vaccine approved for avian flu.
- 2007: Scientists discover how to use human skin cells to create embryonic stem cells.
- 2008: Chemists in Japan create the first DNA molecule made almost entirely of artificial parts.
- 2009: Global biotech crop acreage reaches 330 million acres.
- In 2009, Sasaki and Okana produced transgenic marmosets that glow green in ultraviolet light (and pass the trait to their offspring).
- 2009: FDA approves the first genetically engineered animal for production of a recombinant form of human antithrombin.

- In 2010, Craig Venter was successful in demonstrating that a synthetic genome could replicate autonomously.
- 2010: Dr. J. Craig Venter announces completion of "synthetic life" by transplanting synthetic genome capable of self-replication into a recipient bacterial cell.
- 2010: Harvard researchers report building "lung on a chip" – technology.
- In 2010, scientists created malaria-resistant mosquitoes.
- 2011: Trachea derived from stem cells transplanted into human recipient.
- 2011: Advances in 3-D printing technology lead to "skin-printing."
- 2012: For the last three billion years, life on Earth has relied on two information-storing molecules, DNA and RNA. Now there's a third: XNA, a polymer synthesized by molecular biologists Vitor Pinheiro and Philipp Holliger of the Medical Research Council in the United Kingdom. Just like DNA, XNA is capable of storing genetic information and then evolving through natural selection. Unlike DNA, it can be carefully manipulated.
- 2012: Researchers at the University of Washington in Seattle announced the successful sequencing of a complete fetal genome using nothing more than snippets of DNA floating in its mother's blood.
- 2013: Two research teams announced a fast and precise new method for editing snippets of the genetic code. The so-called CRISPR system takes advantage of a defense strategy used by bacteria.
- 2013: Researchers in Japan developed functional human liver tissue from reprogrammed skin cells.
- 2013: Researchers published the results of the first successful human-to-human brain interface.
- 2013: Doctors announced that a baby born with HIV had been cured of the disease.
- 2014: Researchers showed that blood from a young mouse can rejuvenate an old mouse's muscles and brain.
- 2014: Researchers figured out how to turn human stem cells into functional pancreatic β cells—the same cells that are destroyed by the body's own immune system in type 1 diabetes patients.
- 2014: All life on Earth as we know it encodes genetic information using four DNA letters: A, T, G, and C. Not anymore! In 2014, researchers created new DNA bases in the lab, expanding life's genetic code and opening the door to creating new kinds of microbes.
- 2014: For the first time ever, a woman gave birth to a baby after receiving a womb transplant.
- 2014: An international team of scientists reconstructed a synthetic and fully functional yeast chromosome. A breakthrough seven years in the making, the remarkable advance could eventually lead to custom-built organisms (human organisms included).
- 2014 & Ebola: Until this year, ebola was merely an interesting footnote for anyone studying tropical diseases. Now it's a global health disaster. But the epidemic started at a single point with one human-animal interaction — an interaction which has now been pinpointed using genetic research. A total of 50 authors contributed to the paper announcing the discovery, including five who died of the disease before it could be published.
- 2014: Doctors discovered a vaccine that totally blocks infection altogether in the monkey equivalent of the disease — a breakthrough that is now being studied to see if it works in humans.

- 2015: Scientists from Singapore's Institute of Bioengineering and Nanotechnology designed short strings of peptides that self-assemble into a fibrous gel when water is added for use as a healing nanogel.
- 2015 & CRISPR: scientists hit a number of breakthroughs using the gene-editing technology CRISPR. Researchers in China reported modifying the DNA of a nonviable human embryo, a controversial move. Researchers at Harvard University inserted genes from a long-extinct woolly mammoth into the living cells — in a petri dish — of a modern elephant. Elsewhere, scientists reported using CRISPR to potentially modify pig organs for human transplant and modify mosquitoes to eradicate malaria.
- 2015: Researchers in Sweden developed a blood test that can detect cancer at an early stage from a single drop of blood.
- 2015: Scientists discovered a new antibiotic, the first in nearly 30 years, that may pave the way for a new generation of antibiotics and fight growing drug-resistance. The antibiotic, teixobactin, can treat many common bacterial infections, such as tuberculosis, septicaemia, and C. diff.
- 2015: A team of geneticists finished building the most comprehensive map of the human epigenome, a culmination of almost a decade of research. The team was able to map more than 100 types of human cells, which will help researchers better understand the complex links between DNA and diseases.
- 2015: Stanford University scientists revealed a method that may be able to force malicious leukemia cells to change into harmless immune cells, called macrophages.
- 2015: Using cells from human donors, doctors, for the first time, built a set of vocal cords from scratch. The cells were urged to form a tissue that mimics vocal fold mucosa – vibrating flaps in the larynx that create the sounds of the human voice.
- 2016: A little-known virus first identified in Uganda in 1947—Zika—exploded onto the international stage when the mosquito-borne illness began spreading rapidly throughout Latin America. Researchers successfully isolated a human antibody that "markedly reduces" infection from the Zika virus.
- 2016: CRISPR, the revolutionary gene-editing tool that promises to cure illnesses and solve environmental calamities, took a major step forward this year when a team of Chinese scientists used it to treat a human patient for the very first time.
- 2016: Researchers found that an ancient molecule, GK-PID, is the reason single-celled organisms started to evolve into multicellular organisms approximately 800 million years ago.
- 2016: Stem Cells Injected Into Stroke Patients Re-Enable Patient To Walk.
- 2016: Cloning does not cause long-term health issues, study finds
- 2016: For the first time, bioengineers created a completely 3D-printed 'heart on a chip.'
- 2017: Researchers at the National Institute of Health discovered a new molecular mechanism that might be the cause of severe premenstrual syndrome known as PMDD.
- 2017: Scientists at the Salk Institute in La Jolla, CA, said they're one step closer to being able to grow human organs inside pigs. In their latest research they were able to grow human cells inside pig embryos, a small but promising step toward organ growth.
- 2017: First step taken toward epigenetically modified cotton.

- 2017: Research reveals different aspects of DNA demethylation involved in tomato ripening process.
- 2017: Sequencing of green alga genome provides blueprint to advance clean energy, bioproducts.
- 2017: Fine-tuning 'dosage' of mutant genes unleashes long-trapped yield potential in tomato plants.
- 2017: Scientists engineer disease-resistant rice without sacrificing yield.
- 2017: Blood stem cells grown in lab for the first time.
- 2017: Researchers at Sahlgrenska Academy – part of the University of Gothenburg, Sweden – generated cartilage tissue by printing stem cells using a 3D-bioprinter.
- 2017: Two-way communication in brain-machine interface achieved for the first time.

Scope of Biotechnology:

Biotechnologists can work for various organisations/industries under these positions:

- Medical scientists,
- Biological technicians
- Medical and Clinical Lab Technologists & Technicians
- Biochemists and Biophysicists
- Biomedical Engineers
- Microbiologists
- Epidemiologists
- R&D and Process Development Scientists
- Biomanufacturing Specialists
- Bioproduction Operators

Application of Biotechnology:

1. Nutrient Supplementation

One of the biggest uses of biotechnology is the infusion of nutrients into food in situations such as aid. Therefore, it provides food with heavy nutrients that are necessary in such situations. An example of this application is the production *Golden Rice* where the rice is infused with beta-carotene. The rice has Vitamin A, which the body can easily synthesise.

2. Abiotic Stress Resistance

There is actually very little land on earth that is arable with some estimates place it at around 20 percent. With an increase in the world's population, there is a need for the food sources available to be as effective as possible to produce as much food in as little space as possible. There is also need to have the crops grown to be able to make use of the less arable regions of the world.

This means that there is a need to develop crops that can handle these abiotic stresses such as salinity, drought and frost from cold. In Africa and the Middle East, for instance, where the climate can be

unforgiving, the practice has played a significant role in the development of crops that can withstand the prevailing harsh climates.

3. Industrial Biotechnology

The industrial applications of biotechnology range from the production of cellular structures to the production of biological elements for numerous uses. Examples include the creation of new materials in the construction industry, and the manufacture of beer and wine, washing detergents, and personal care products.

4. Strength Fibres

One of the materials with the strongest tensile strength is spider webs. Amongst other materials with the same cross sectional width, spider webs can take more tensional force before breaking than even steel. This silk has created a lot of interest with the possible production of materials made from silk including body armour such as bullet proof jackets. Silk is used because it is stronger than Kevlar (the material most commonly used to make body armour).

Biotechnological techniques have been used to pick the genes found in spiders and their infusion in goats to produce the silk proteins in their milk. With this initiative, it make production easier as goats are much easier to handle compared to spiders and the production of silk via milk also help make the processing and handling much easier compared to handling the actual silk strands.

5. Biofuels

One of the biggest applications of biotechnology is in the energy production sector. With fears over the dwindling oil resources in the world and their related environmental impacts, there is a need to protect the globe's future by finding alternative environmentally friendly fuel sources. Biotechnology is allowing this to happen with advances such as using corn to produce combustible fuel for running car engines. These fuels are good for the environment as they do not produce the greenhouse gases.

6. Healthcare

Biotechnology is applied in the healthcare sector is the development of pharmaceuticals that have proven problematic to produce though other conventional means because of purity concerns.

Production of Antibiotics:

- **Antibiotic**: Any substance that can destroy or inhibit the growth of bacteria and similar microorganisms.

- **Fermentation**: Any of many anaerobic biochemical reactions in which an enzyme (or several enzymes produced by a microorganism) catalyses the conversion of one substance into another; especially the conversion (using yeast) of sugars to alcohol or acetic acid with the evolution of carbon dioxide.

- **Metabolite**: Any substance produced by, or taking part in, a metabolic reaction.

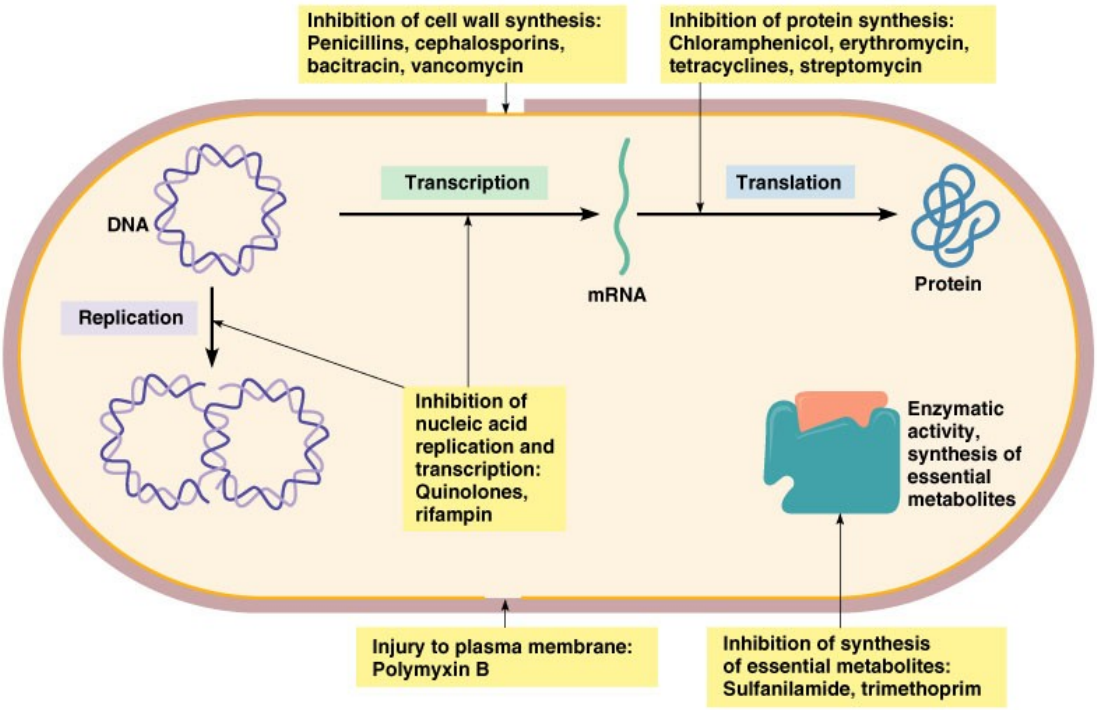
Antibiotic/Antimicrobial Compounds:

- **Antibiotic:** Chemical produced by a microorganism that kills or inhibits the growth of another microorganism
- **Antimicrobial agent:** Chemical that kills or inhibits the growth of microorganisms

Microbial Sources of Antibiotics:

Microorganism	Antibiotic
Gram-Positive Rods	
Bacillus subtilis	Bacitracin
Bacillus polymyxa	Polymyxin
Actinomycetes	
Streptomyces nodosus	Amphotericin B
Streptomyces venezuelae	Chloramphenicol
Streptomyces aureofaciens	Chlortetracycline and tetracycline
Streptomyces erythraeus	Erythromycin
Streptomyces fradiae	Neomycin
Streptomyces griseus	Streptomycin
Micromonospora purpureae	Gentamicin
Fungi	
Cephalosporium spp.	Cephalothin
Penicillium griseofulvum	Griseofulvin
Penicillium notatum	Penicillin

Mode of Antimicrobial Action:



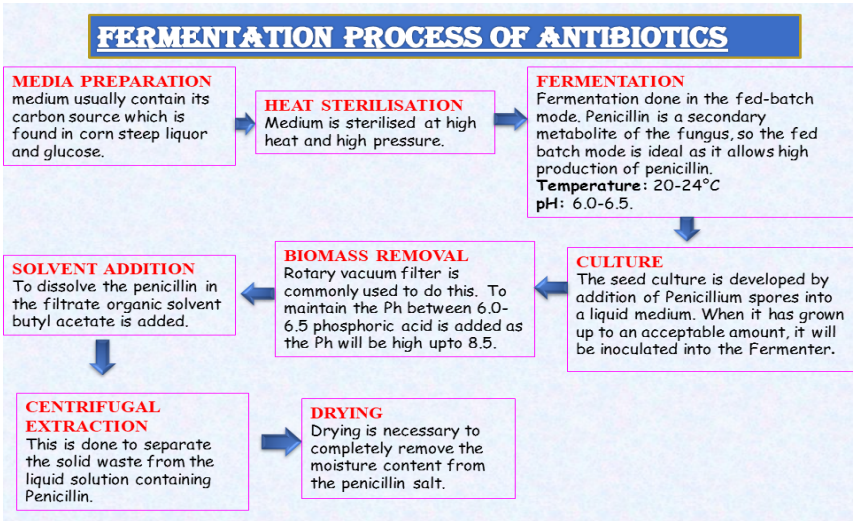
Industrial production techniques

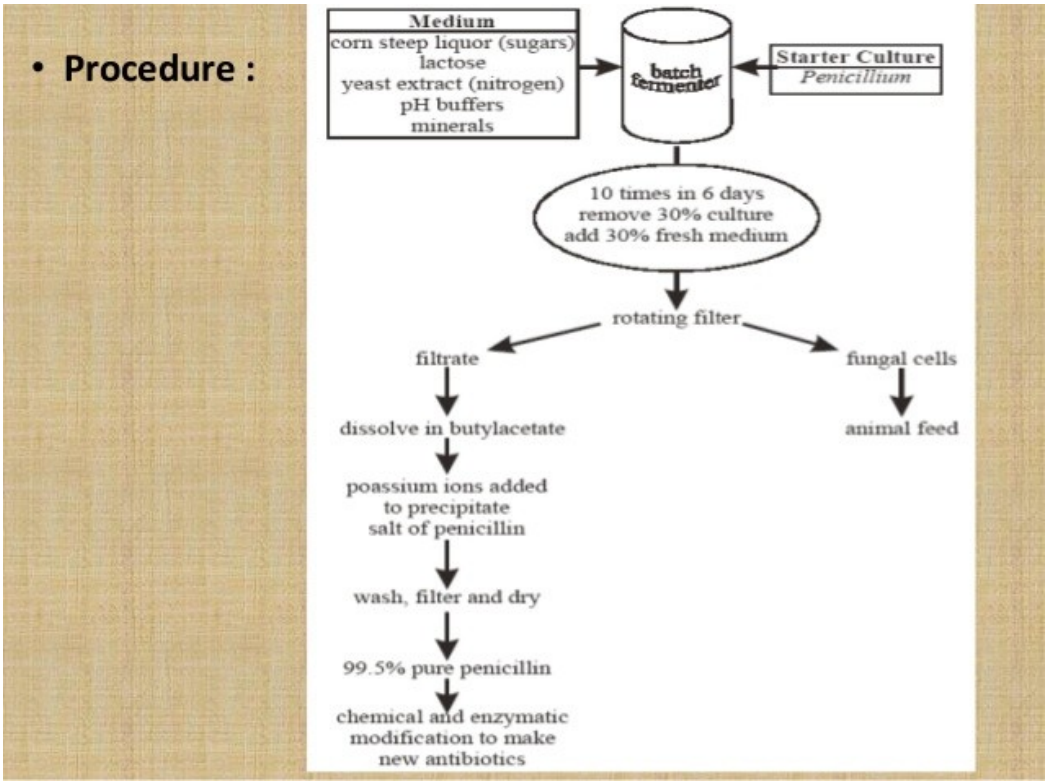
1. **Fermentation**
   - Industrial microbiology can be used to produce antibiotics via the process of fermentation, where the source microorganism is grown in large containers (100,000–150,000 liters or more) containing a liquid growth medium.
   - Oxygen concentration, temperature, pH and nutrient levels must be optimal, and are closely monitored and adjusted if necessary.
   - As antibiotics are secondary metabolites, the population size must be controlled very carefully to ensure that maximum yield is obtained before the cells die.
   - Once the process is complete, the antibiotic must be extracted and purified to a crystalline product.
   - This is easier to achieve if the antibiotic is soluble in organic solvent. Otherwise it must first be removed by ion exchange, adsorption or chemical precipitation.
2. **Semi-synthetic**
   - A common form of antibiotic production in modern times is semi-synthetic.

- Semi-synthetic production of antibiotics is a combination of natural fermentation and laboratory work to maximize the antibiotic.
- Maximization can occur through efficacy of the drug itself, amount of antibiotics produced, and potency of the antibiotic being produced.
- Depending on the drug being produced and the ultimate usage of said antibiotic determines what one is attempting to produce.
- An example of semi-synthetic production involves the drug ampicillin. A beta lactam antibiotic just like penicillin, ampicillin was developed by adding an addition amino group (NH_2) to the R group of penicillin.
- This additional amino group gives ampicillin a broader spectrum of use than penicillin. Methicillin is another derivative of penicillin and was discovered in the late 1950s, the key difference between penicillin and methicillin being the addition of two methoxy groups to the phenyl group.
- These methoxy groups allow methicillin to be used against penicillinase producing bacteria that would otherwise be resistant to penicillin.

3. Synthetic

- Not all antibiotics are produced by bacteria; some are made completely synthetically in the lab.
- These include the quinolone class, of which nalidixic acid is often credited as the first to be discovered.
- Like other antibiotics before it the discovery of nalidixic acid has been chalked up to an accident, discovered when George Lesher was attempting to synthesize chloroquine.





Uses of Antibiotics:

- Antitumor antibiotics
- Food preservative antibiotics
- Antibiotics used in animal feed and veterinary medicine
- Antibiotics for control of human and plant diseases
- Antibiotics as tools in molecular biology

Production of Insulin:

Insulin is a hormone that is responsible for allowing glucose in the blood to enter cells, providing them with the energy to function. A lack of effective insulin plays a key role in the development of diabetes.

- **Source:** Beta cells of Islets of Langerhens.
- **Chemistry:** Peptide-51 AA, A Chain 21 AA, B chain 30 AA.
- **MW:** 5808.(First high MW hormone to be by the G.Eng)
- **Half life:** 5-10 Min.
- **Fate:** Endocytosis ㄱ & Proteolysis (80% in liver & kidney).
- **Species Specificity:** Bovine ㄱ & Human.
- **Prolong action:** Zn & Protamine.

- It exist in Zn crystals as hexamers & monomer.
- When diluted in the circulation , it exist as monomer.

Why E.coli ?

- Cows & pig – immunogenic.
- Yeast cells –costly.
- Plant cells – not fully developed.
- *E.coli* – Simple, well-understood genetics.
    - It's very easy to manipulate.
    - Culturing cost is minimal.
    - High level of expression.
    - Fermentation easy to scale up.
    - Inclusion bodies may be easy to purify.

Methods of insulin production:

- There two main methods exits for the production of recombinant human insulin from genetically modified bacterial cultures:
    1. Two chain method (both A & B chain are synthesized by separate E. coli plasmid)
    2. Proinsulin method (intracellular or secreted) - The proinsulin method is currently the most efficient method because, single isolation & isolation steps involved.

Recombinant DNA, or rDNA, is DNA which specifically encodes a protein.

This is cut from genomic DNA by a restriction enzyme which cuts DNA at specific sequences along the chain.

These pieces are then analyzed and the DNA needed to make the protein is extracted and purified.

Since insulin contains two polypeptide chains linked by disulfide bonds, two pieces of DNA are extracted.

These DNA strands are then placed into two different plasmids

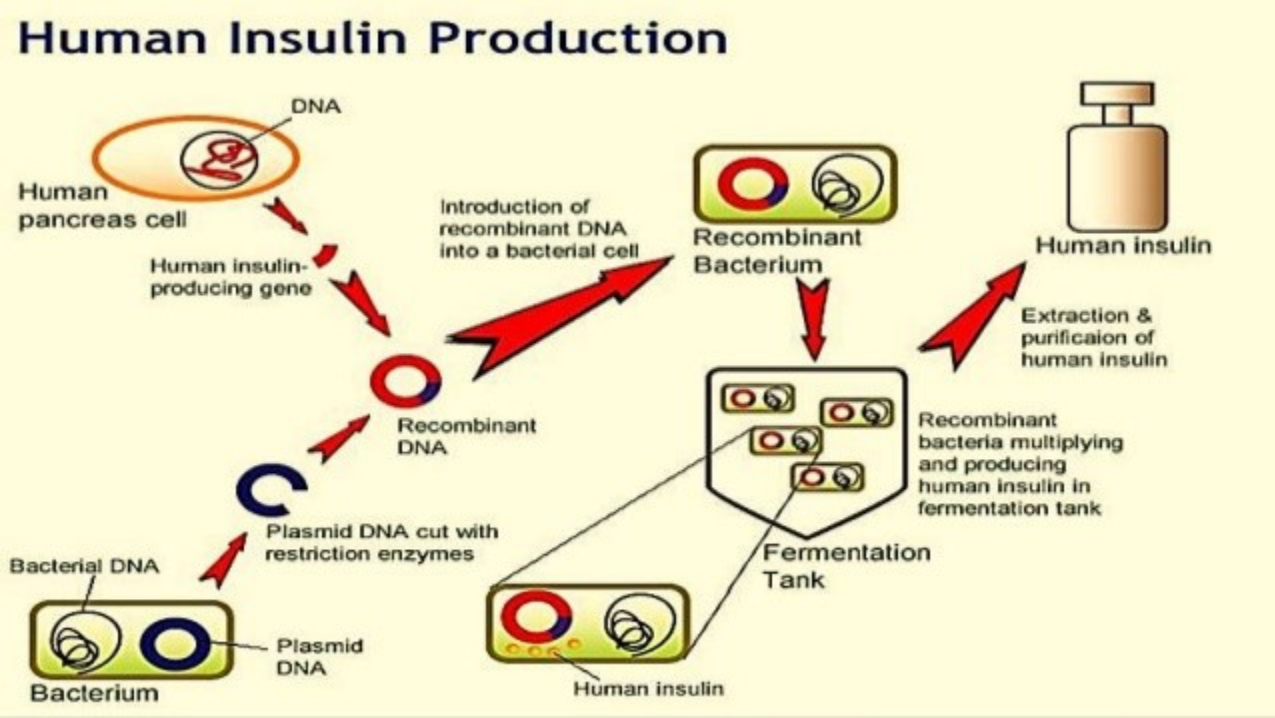
These plasmids are also cut with the same restriction enzymes as the DNA, which will allow the DNA to be fixed into the plasmid by DNA ligase.

The plasmids are then incubated with a weakened strain of *E. coli.*

Since only some of the bacteria will take up the plasmid, a gene encoding an enzyme which breaks down a certain antibiotic is also included in the plasmid, which allows bacteria with the plasmid to grow on a plate containing the antibiotic while the other bacteria die.

These bacteria are then allowed to grow and replicate, which allows the plasmid and the insulin gene to replicate millions of times.

Then the bacteria are given a signal to produce the protein, and insulin identical to that of humans can be produced and purified.



If you don't have diabetes, insulin helps:

- **Regulate blood sugar levels.** After you eat, carbohydrates break down into glucose, a sugar that is the body's primary source of energy. Glucose then enters the bloodstream. The pancreas responds by producing insulin, which allows glucose to enter the body's cells to provide energy.
- **Store excess glucose for energy.** After you eat — when insulin levels are high — excess glucose is stored in the liver in the form of glycogen. Between meals — when insulin levels are low — the liver releases glycogen into the bloodstream in the form of glucose. This keeps blood sugar levels within a narrow range.

If you have diabetes:

Your glucose levels will continue to rise after you eat because there's not enough insulin to move the glucose into your body's cells. People with type 2 diabetes don't use insulin efficiently (insulin resistance) and don't produce enough insulin (insulin deficiency). People with type 1 diabetes make little or no insulin.

Untreated, high blood glucose can eventually lead to complications such as blindness, nerve damage and kidney damage.

Streptokinase Enzyme:

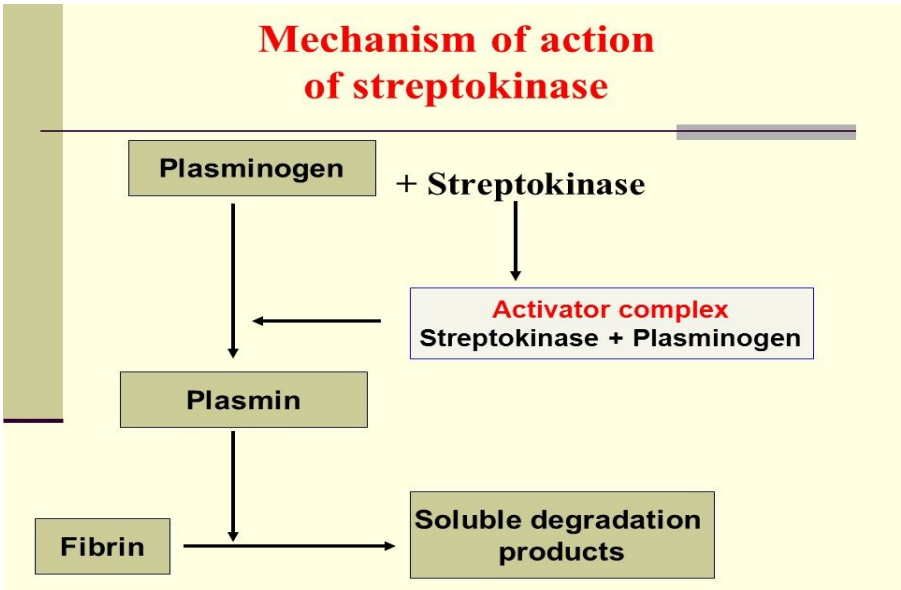
- **Streptokinase** (**SK**) is a thrombolytic medication and enzyme.
- As a medication it is used to break down clots in some cases of myocardial infarction (heart attack), pulmonary embolism, and arterial thromboembolism.
- The type of heart attack it is used in is an ST elevation myocardial infarction (STEMI).
- It is given by injection into a vein.
- Streptokinase was discovered in 1933 from beta-hemolytic streptococci

Introduction:

•A blood clot (thrombus) developed in the circulatory system can cause vascular blockage leading to serious consequences including death.

• A healthy hemostatic system suppresses the development of blood clots in normal circulation, but reacts extensively in the event of vascular injury to prevent blood loss.

- Outcomes of a failed homeostasis include stroke, pulmonary embolism, deep vain thrombosis and acute myocardial infraction.



Streptococcal fibrinolysis:

- These enzyme activates a fibrinolytic enzyme in human serum, which splits fibrin into smaller fragments, thus it causes rapid dissolution of blood clots and fibrinous exudates.
- Therefore, streptokinase acts indirectly upon a substrate of fibrin or fibrinogen.

Source:

- This enzyme is produced by certain bacteria. The most frequently employed for manufacture are: haemolytic streptococci, particularly those of the lancefied groups A, human C and G. Steptodornase is a related enzyme, which act directly upon a substrate of deoxyribonucleoproteins (DNA)

Manufacturing process:

- It is produced by fermentation process which involves the following steps:-

1. Preparation of medium
2. Fermentation
3. Purification of the product.

1. Preparation of medium:

Ingredients:

i). Casein digests solution: It is prepared by dissolving casein in water in specified proportion. It is heated to 100 C and maintained the same temperature, till the solution is clear. The resultant solution is rapidly cooled to 15 C and filtered through a coarse filter paper.

- **Toluene** in small quantity is added for the purpose of preservation. It is stored for four days at 20 C and filtered to remove insoluble material.

ii). Dextrose (a carbohydrate source)

iii). Amino acids : a)Cysteine in 10%HCl b)Glycine c)Tryptophan.

iv). Vitamins a)Thiamine hydrocloride b)Riboflavin c)Nicotinic acid d)Pyridoxine e)Calcium pentothenate

v). Trace elements – $MgSO_4$, $CuSO_4$, $MnSO_4$, $FeSO_4$

vi). Potassium bicarbonate

vii). Potassium dihydrogen phosphate

viii). Uracil

ix). Adenyl sulphate

x). Thioglycolic acid

2. **Fermentation:**

- Sterilized medium is inoculated with seed inoculation of bacterium; ***S. haemolyticus*** having a bacterial count of 20 billions/ml.
- Fermentation is carried out in a tank for 14hrs at 37C.
- During this period no pH adjustment, aeration or modification are made.
- Later, dextrose 50% is added and pH is adjusted to 6 at 15 min. interval with 5N sodium hydroxide.
- After each adjustment of pH, 50% dextrose is added.
- Fermentation is continued till bacterial count ceases to increase(about 3 hrs).
- At this stage fermentation medium contains appx. 1000 units/ml

3. **Purification:**

- Crude streptokinase is first dialysed against phosphate buffer then it is applied on modified cellulosic columns and eluted with phosphate buffer with increasing pH and molarity(increasing pH 5.8 to 8.5 and molarity 0.005to 0.1 M).
- pH and molarity are the important factors in purification.
- At 0.1 M: streptokinase is eluted
- > 0.1 M: improper adsorption and separation pH
- > 8.5:adsorption capacity of cellulose decreases
- < 5.8:streptokinase is precipitated
- At pH 8 and molarity 0.75, impurities are eluted.

Purification by deae cellulose: Crude streptokinase, phosphate buffer 0.2 M and DEAE cellulose in the proportion of 3:2:1 are stirred for 1 hour and filtered. Cake is washed with phosphate buffer 0.025 m by stirring for 30 mins. It is again filtered and cake is suspended in 0.1 m phosphate buffer by stirring for 1 hour. Filterate is collected which contains pure streptokinase.

Uses: Treatment of thromboembolic disorders for the lysis of pulmonary emboli, arterial thrombus, deep vein thrombus and acute coronary artery thrombosis.

Recombinant Hepatitis B Vaccine:

Hepatitis B Vaccine is indicated for prevention of infection caused by all known subtypes of hepatitis B virus

Hepatitis B Recombinant Vaccine It's a novel and significant developed vaccine which is produced from the antigenic proteins of Hepatitis B virus by recombinant process that duplicates the chemical messages and secreted factors (Interleukin-2) for the communication and activity of immune cells.

General steps for Recombinant Hepatitis B Vaccine production:

Vaccine production Production of these genes is needed in order to get production of vaccines on a large scale. A general procedure for the production of recombinant Hepatitis B vaccines are described here-

1. HBs antigen producing gene is isolated from the HB virus by normal isolation process (cell lysis, protein denaturation, precipitation, centrifugation and drying).

2. A plasmid DNA is extracted from a bacterium- E.coli and is cut with restriction enzyme- Eco RI forming the plasmid vector

3. The isolated HBs antigen producing gene is located and inserted into the bacterial plasmid vector on forming the recombinant DNA.

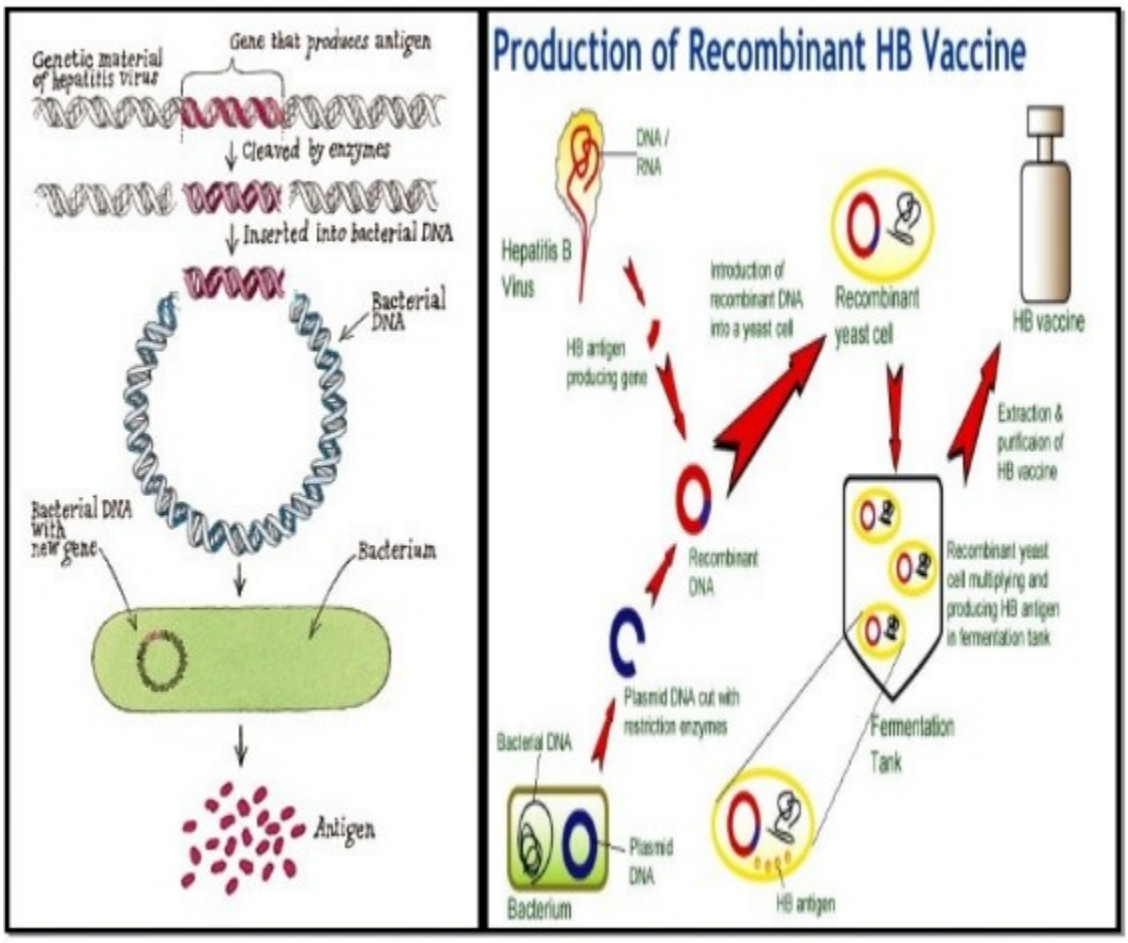
4. This recombinant DNA, containing the target gene, is introduced into a yeast cell forming the recombinant yeast cell.

5. The recombinant yeast cell multiplies in the fermentation tank and produces the HBs antigens.

6. After 48 hours, yeast cells are ruptured to free HBsAg. The mixture is processed for extraction.

7. The HBs antigens are purified.

8. HBsAg are combined with preserving agent and other ingredients and bottled. Now it is ready for vaccination in humans.



Uses:

This vaccine is used to help prevent infection from the hepatitis B virus. Hepatitis B infection can cause serious problems including liver failure, persistent hepatitis B infection, cirrhosis, and liver cancer. Preventing infection can prevent these problems.

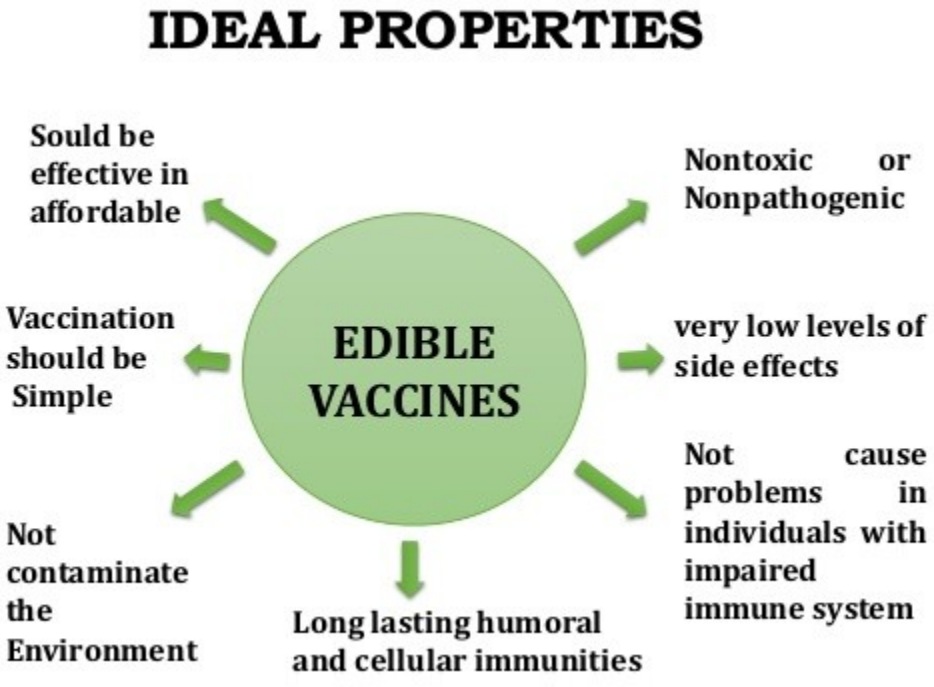
Hepatitis B vaccine is a genetically engineered (man-made in the laboratory) piece of the virus. It does not contain live virus, so you cannot get hepatitis from the vaccine. This vaccine works by helping the body produce immunity (through antibody production) that will prevent you from getting infection from hepatitis B virus. Hepatitis B vaccine does not protect you from other virus infections (such as HIV virus which causes AIDS; hepatitis A, hepatitis C or hepatitis E; HPV virus which causes genital warts and other problems).

The vaccine is recommended for people of all ages, especially those at an increased risk of getting the infection. Those at an increased risk include health care personnel, laboratory workers who handle blood and patient specimens, police, fire and emergency medical personnel who give first aid treatment, hemophiliacs, dialysis patients, people who live with or spend much time with people with persistent hepatitis B infections,

people with multiple sex partners, men who have sex with men, sex workers, injection drug abusers, and people.

Edible vaccines:

- The phrase **edible vaccines** was first used by Charles Arntzen in 1990 and refers to any foods; typically plants, that produce vitamins, proteins or other nourishment that act as a vaccine against a certain disease.
- Once the plant, fruit, or plant derived product is ingested orally, it stimulates the immune system.
- Specifically, it stimulates both the mucosal and humoral immune systems.
- Edible vaccines are genetically modified crops that contain added "immunity" for specific diseases.
- Edible vaccines offer many benefits over traditional vaccines, due to their lower manufacturing cost and a lack of negative side effects.
- However, there are limitations as edible vaccines are still new and developing.
- Edible vaccines are currently being developed for measles, cholera, foot and mouth disease, Hepatitis B and Hepatitis C.



Concept of edible vaccine:

Developed by Arntzen in the 1990s. Introduce genes of interest into plants (Transformation)

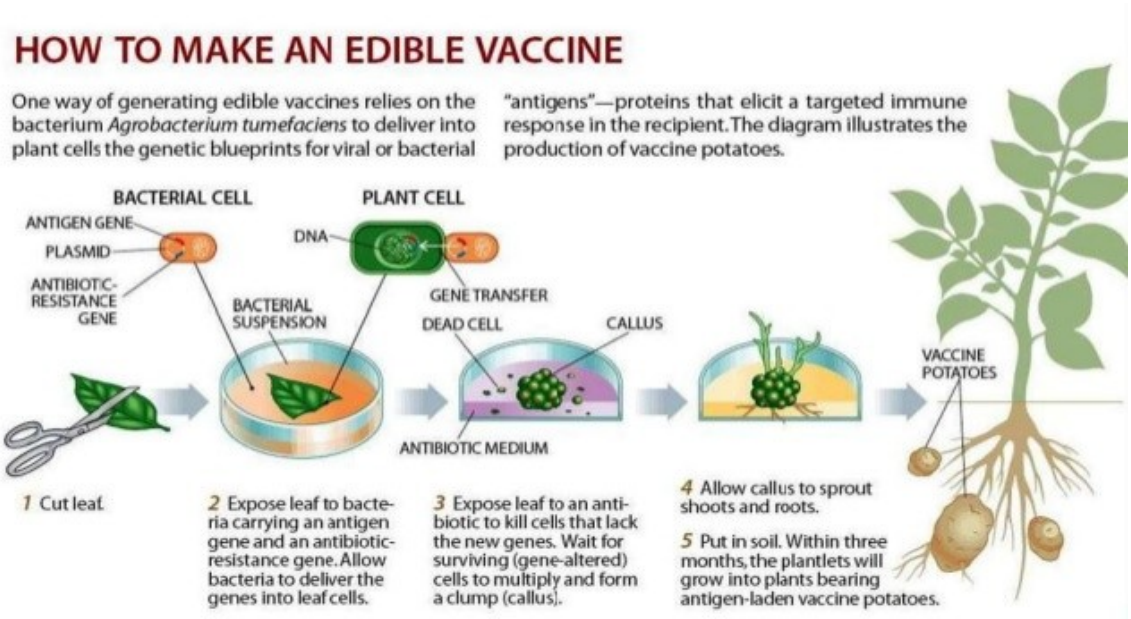
Genes expressed in the plant tissues edible parts (Transgenic plants)

⇩

Genes encode putatively protective vaccine antigens from viral, bacterial, and parasitic pathogens that cause disease in humans and animals

⇩

Ingestion of the edible part of the transgenic plant (Oral delivery of vaccine)



Advantages of Edible vaccines:

- DO not require administration by injection.
- Possible production of vaccines with low costs.
- Do not require separation and purification ofϖ vaccines from plant materials.
- Necessary syringeϖ & needles not required.
- Economical in mass production andϖ transportation. Heat stable, eliminating the need for refrigeration.

Disadvantage of edible vaccine:

- Development of immunotolerance to vaccine peptide or protein.
- Consistency of dosage form fruit to fruit, plant-to-plant, and generation- to-generation is not similar.
- Stability of vaccine in fruit is not known.
- Dosage of vaccines would be variable.

- Selection of best plant is difficult.
- Certain foods like potato are not eaten raw, and cooking the food might weakens the medicine present in it.
- Not convenient for infants.

Monoclonal antibodies production:

1. **Immunization:**

Immunization is necessary to present an antigen in a suitable form to induce the most vigorous humoural immune response to an animal. This essential step will result in the production of cells secreting antibody against your chosen antigen

2. **Hybridoma Production:**

A hybridoma is a cell line arising from one hybrid cell that is capable of secreting a monoclonal antibody specific to one epitope of your antigen permanently in culture. The hybrid cell is produced through the fusion of specific antibody producing B-cell from an immunized animal.

Production of a mouse hybrid cell:

- During the fusion process, B cells are isolated from the mouse spleen, mixed with the mouse myeloma cell line and fusion is induced with polyethylene glycol.
- The resulting hybridomas are then cultured in tissue culture medium containing Hypoxathine, Aminopterin, Thymidine (HAT), a step which kills any unfused myeloma cells that might outgrow the other weaker hybridoma cells.
- Unfused B cells have limited powers of division and will die off naturally in culture.
- Ten days after the fusion process, culture supernatant is collected and tested for the presence of the desired antibody.

3. **Cloning:**

- The objective of cloning the cells producing the antibody of interest is to ensure that the desired hybridoma cell line produced is obtained from a single fused cell.

- After a fusion, many different hybrid cells will be present in a single well resulting in the growth of multiple colonies in each well.

- Your specific antibody-secreting colony is therefore likely to be mixed with other cells that are either non-secreting or which are producing an antibody of undesired specificity.

4. Freezing and Thawing of cell line:

Hybridoma and myeloma cell lines are stored by freezing the cells at a controlled rate (approximately 1ºC per minute) in an appropriate cryoprotectant. This procedure allows the cell line to be preserved indefinitely.

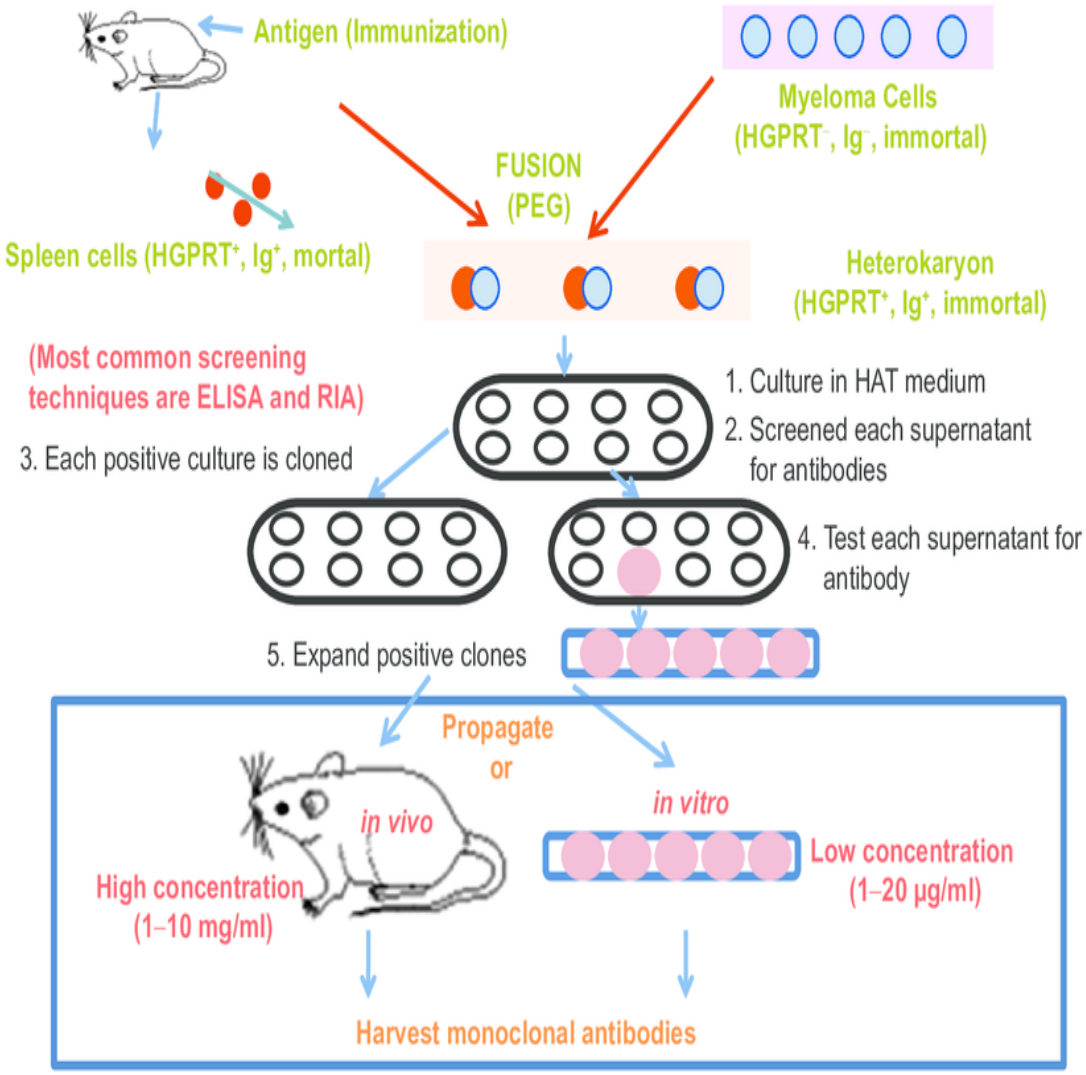
Freezing Method

- Only cells that are healthy and rapidly dividing should be frozen.
- One or two days before freezing, split the cells 1:10 into fresh medium and maintain in culture.
- On the day of freezing, count the cells.
- You need to have between 2 to $5x10^6$ cells in each freezing vial.
- Transfer the appropriate volume of cells to a sterile centrifuge tube.
- Spin at 300g for 5 minutes.
- Carefully remove as much supernatant as possible without disturbing the pellet.
- Gently resuspend the cell pellet in 1.5ml of freezing medium containing 10% Dimethylsulphoxide (DMSO, from Sigma D2650) in 90 % FCS previously heat-inactivated).
- Transfer 1.5ml of the resulting cell suspension into a freezing vial (Thermo 375418).
- Seal the vial (finger tight) to prevent liquid nitrogen from entering the vial.
- Place the sealed vials in a special freezing container (Thermo Scientific 5100-0001) which allows the cells to cool down slowly.
- Close the freezing container and place at -80ºC for at least 4 hours.
- Transfer the vials of frozen cells to liquid nitrogen.
- Extended storage of cells at -80ºC is not recommended.

- Prolonged exposure to DMSO is toxic to cells. For example, handling more than 10-20 vials at any one time will lead to extended exposure of the cells to DMSO prior to freezing.

Thawing Method

- Remove the frozen vial from the liquid N2 storage.
- Loosen the cap of the vial slightly to release the pressure inside.
- Thaw the cells in a 37ºC water bath.
- Keep the lid of the freezing vial above the surface of the water to lessen the chances of contamination.
- When the cells are almost thawed (only a small piece of ice) move the vial to the tissue culture hood.
- Wipe the outside of the vial with 70% ethanol and remove the top.
- Carefully remove the cell suspension using a sterile Pasteur pipette.
- Transfer the contents to a centrifuge tube containing 10 ml of appropriate culture médium (See Appendix I – remember myeloma medium should not contain HAT).
- Spin the cell suspension gently at 300g for 5 min.
- Carefully remove the medium without disturbing the pellet.
- Gently resuspend the cells in 10ml of fresh appropriate culture medium and place in a small T25 flask (Corning 3056).
- Take 1ml from this flask and add to 9mls of complete culture medium in another small flask. This step ensures that at least one concentration of cells is suitable for continued culture.
- Place the flasks in a 5% CO_2 incubator with their tops loosened enabling gaseous exchange to occur.



Applications of Monoclonal Antibodies

1. **Isolation and purification:** Monoclonal antibodies can be used to purify individual molecule from a mixture even when they are present in low concentration, e.g. interferon and coagulation factor VIII.
2. **Identification of cells and clones:** For example T_H, and T_C cells are identified by using anti-CD4 and anti-CD8 mAb.
3. **Diagnostic reagents:** The antigen detection kits employ various mAb tagged with detection molecules, such as fluorescent dye or enzyme to detect the specific antigens in the clinical specimen such as:

- **Detection of infections**, such as hepatitis B, serogrouping of streptococci, etc.
- **Pregnancy detection test**-by using monoclonal antibody against human chorionic gonadotropin.

- **Blood grouping** can be done by using anti-A and anti-B monoclonal antibodies.
- **Tumor detection** and imaging: By using mAb specific for tumor antigens secreted by tumor cells (e.g. prostate-specific antigen).
- **Tissue typing** for transplantation can be done by using anti-HLA monoclonal antibodies.

4. **Monitoring** proteins and drug levels in serum.
5. **Passive immunity:** For post-exposure prophylaxis against various infections, mAb targeting specific antigens of the infecting organism can be administered. Examples include- immunoglobulins against hepatitis B, rabies, and tetanus.
6. **Therapeutic use:** Monoclonal antibodies are used in the treatment of various inflammatory and allergic diseases and cancers. Monoclonal antibodies (mAbs) are useful to treat some of the cancer types.
7. **Naked mAbs** (antibodies without attached drug or radioactive material) are the most common type of mAbs used to treat cancer. So far, the US FDA has approved more than a dozen mAbs e.g. alemtuzumab, trastuzumab to treat certain cancers. Similarly, basiliximab is used to treat transplant rejection while belimumab treats systemic lupus erythematosus.
8. **Used as immunotoxin: mAb conjugated with bacterial/ chemical toxins** (e.g. diphtheria toxin) can be used to kill the target cells such as cancer cells. Here, mAb against surface receptors helps in binding to the target cells and the toxin helps in target cell killing.
9. **Used as enzymes:** Abzyme is a monoclonal antibody with catalytic activity.

UNIT II:

BIOFERTILIZERS – DEFINITION:

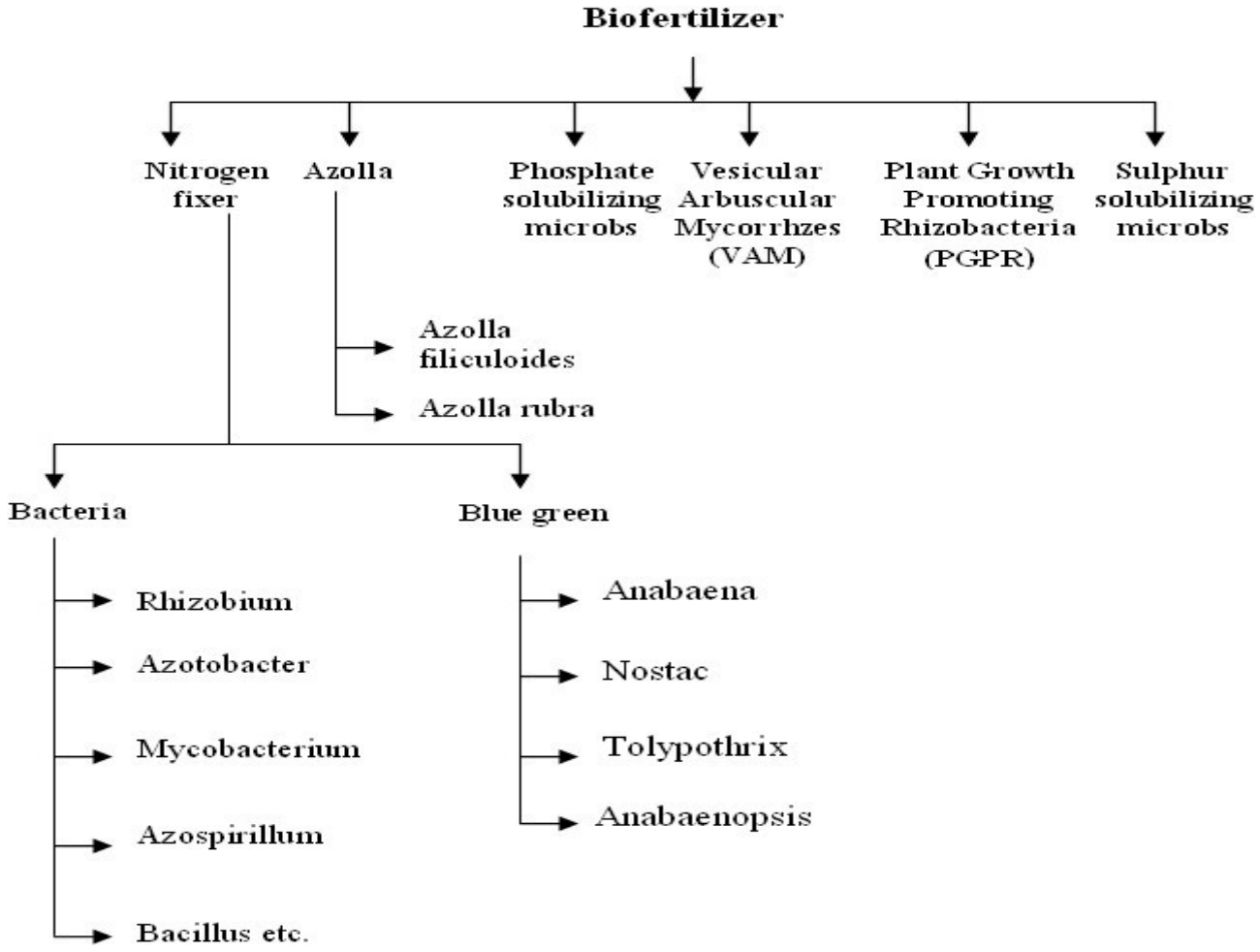
Biofertilizer is a Natural organic fertilizer known that helps to provide all the nutrients required by the plants and helps to increase the quality of the soil with a natural microorganism environment.

- The use of chemical fertilizers and pesticides has caused tremendous harm to the soil, food & fiber products and environment
- Biofertilizers, an environmentally friendly fertilizer now used in most advanced countries
- Biofertilizers are organisms that enrich the nutrient quality of soil
- The main sources of Biofertilizers are bacteria, fungi, and cynobacteria
- The most striking relationship that these have with plants is symbiosis, in which the partners derive benefits from each other.

ADVANTAGES OF BIO-FERTILIZERS

- Cost effective.
- Supplement to fertilizers.
- Eco-friendly (Friendly with nature).
- Reduces the costs towards fertilizers use, especially regarding nitrogen and phosphorus
- Bio-fertilizers improve soil texture and yield of plants
- They do not allow pathogens to flourish
- They do not cause any harm to the environment.

TYPES OF BIOFERTILIZERS:



***Rhizobium* - ISOLATION IDENTIFICATION CHARACTERIZATION AND APPLICATION**

Rhizobium:

- They are Gram-negative
- motile
- non-sporulating rods

ISOLATION OF *Rhizobium:*

Yeast Extract Mannitol Agar (YEMA) plates were prepared and sterilized by autoclaving

⬇

Legume plants (Groundnut) were carefully uprooted and washed under running water to remove the adhesive soil particles

⬇

Healthy unbroken pink nodules were selected

↓

Surface Sterilization of root nodules by 0.1 % mercuric chloride and 3-5% hydrogen peroxides

↓

The nodules were immersed in sterilizing agents for 4-5 minutes and then washed with sterile distilled water.

↓

Then they are washed in 70% ethyl-alcohol followed by washing with sterile distilled water again

↓

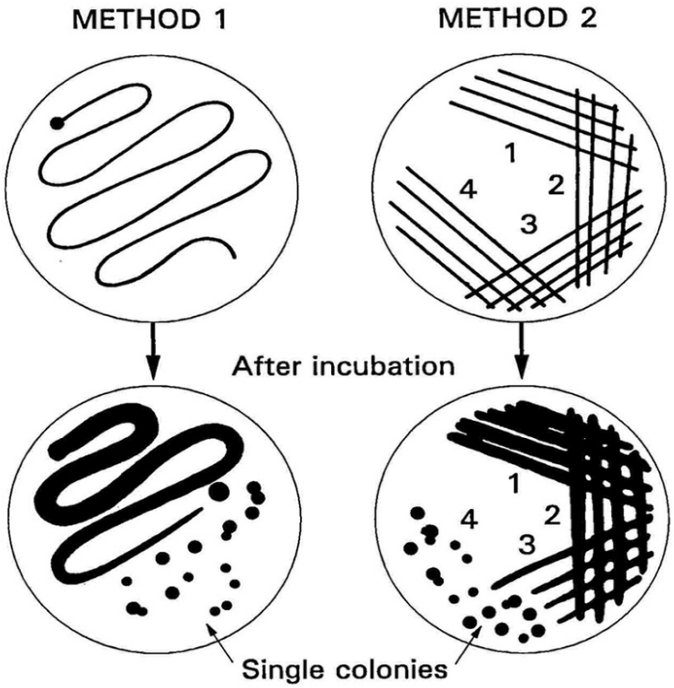
Crushed nodular extract (1 ml) suspension was diluted with 9 ml of sterile distilled water (10^{-1}) making the dilution from 10^{-2} to 10^{-8}

↓

Suspension (0.1 ml) of nodular extract was inoculated into sterile YEMA plates

↓

Inoculated Petri plates were incubated for 4-7 days in an incubator at 37ºC.



IDENTIFICATION AND CHARACTERIZATION OF *Rhizobium*

CELLULAR	
Staining	Gram-negative
Morphology	Rods 0.5-0.9 x 1.2-3.0 um. Commonly pleomorphic under adverse growth conditions
Motility	Motile by one polar or subpolar flagellum or two to six peritrichous flagella
Specialized structures	Fimbriae have been described on a few strains Usually contain granules of poly-B- hydroxyl butyrate which are refractile by phase-contrast microscopy. Non spore forming
Division	
COLONIAL	

Solid surface	Colonies are circular, convex, semi translucent, raised and mucilaginous, usually 2-4 mm in diameter within 3-5 days on yeast-mannitol-mineral salts agar.
Liquid	Pronounced turbidity develops after 2 or 3 days in agitated broth

CLASSIFICATION OF *Rhizobium:*

Rhizobium is not part of a species because it is not a species. *Rhizobium* is the name of a genus of bacteria that fix nitrogen. Inside the genus is a multitude a species. Its name comes from Greek (Riza = Root and Bios = Life). Rhizobim's bacteria that are part of its genus are all aerobic bacteria.

Domain: Eubacteria

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Alpha Proteobacteria

Order: Rhizobiales:

Family: Rhizobicacea

Genus: Rhizobium

Examples of *Rhizobium sp.*

R. alamii, *R. alkalisoli*, *R. cellulosilyticum*, *R. gallicum*, *R. indigoferae*, *R. leguminosarum*, *R. leucaenae*,*R. loessense*, *R. lupini*, *R. mesoamericanum*, *R. nepotum*, *R. oryzae*, *R. petrolearium*, *R. phaseoli*, *R. pisi*

MASS PRODUCTION OF *Rhizobium* BIOFERTILIZER

Methods of Cultivation:

Following are the steps of mass cultivation of *Rhizobium:*

Sterilize the growth medium and inoculate with broth of mother culture prepared in advance

Incubate for 3-4 days at 30 - 32°C

Test the cultures for its purity and transfer to a large fermenter, wait for 4-9 days for bacterial growth (for good bacterial growth make the device for its aeration)

Allow to grow the bacteria either in a large fermenter containing broth or in small flasks as per demand

↓

Check the quality of broth

↓

Blend the broth with sterile carrier *e.g.* peat, lignite, farmyard manure and charcoal powder

↓

Pack the culture in polyethylene bags and keep at 25°C

↓

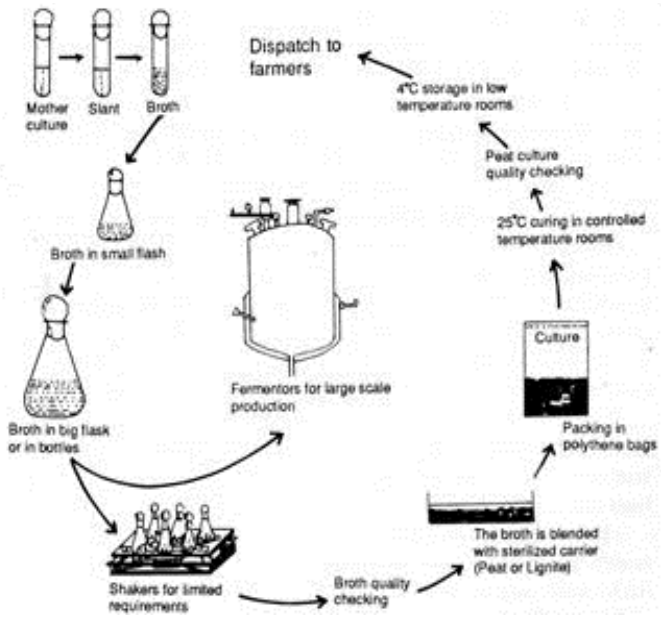
Check the quality of carrier culture

↓

Store at 4°C in a controlled-temperature room

↓

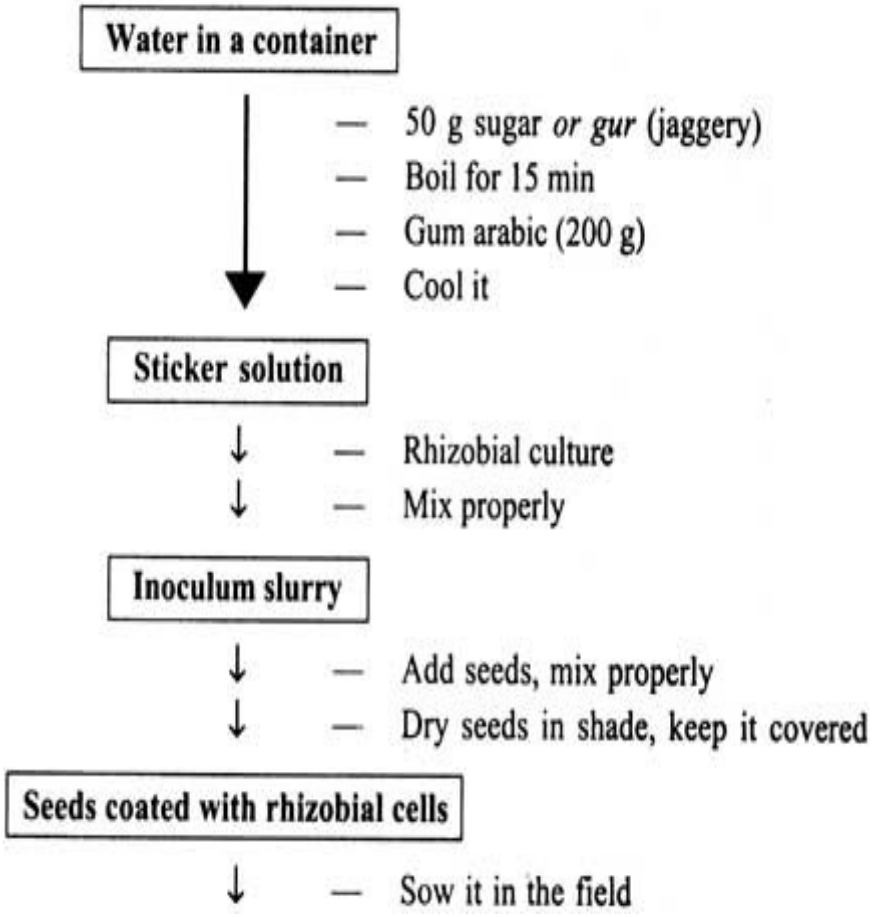
Supply to farmers



METHODS OF SEED INOCULATION WITH RHIZOBIAL CULTURE:

- Dissolve 10 per cent sugar or *gur* (jaggery) in water by boiling it for some time
- Leave the content to cool down. Gum arabic solution (10%) may also be added to the solution
- This serves as sticker for *Rhizobium* cells to seeds
- Mix this carrier based culture of *Rhizobium* to form the inoculum slurry
- For one hectare, 400 g charcoal based culture would be sufficient for mixing the seeds
- Transfer the inoculum slurry on seeds and mix properly

- The number of rhizobial cells/ seed should be between 10^5 to 10^6
- Spread the seeds in shade for drying on cement floor or plastic sheets



APPLICATION OF BIOFERTILIZERS:

1. Seed treatment or seed inoculation
2. Seedling root dip
3. Main field application

Seed treatment

- One packet of the inoculant is mixed with 200 ml of rice kanji to make a slurry
- The seeds required for an acre are mixed in the slurry so as to have a uniform coating of the inoculant over the seeds and then shade dried for 30 minutes
- The shade dried seeds should be sown within 24 hours
- One packet of the inoculant (200 g) is sufficient to treat 10 kg of seeds.

Seedling root dip

- ☆ This method is used for transplanted crops
- ☆ Two packets of the inoculant is mixed in 40 litres of water
- ☆ The root portion of the seedlings required for an acre is dipped in the mixture for 5 to 10 minutes and then transplanted

Main field application

- ❖ Four packets of the inoculant is mixed with 20 kgs of dried and powdered farm yard manure and then broadcasted in one acre of main field just before transplanting.

APPLICATION OF *Rhizobium* BIOFERTILIZER:

- ➢ It is a low cost and easy technique
- ➢ It can be used by small and marginal farmers
- ➢ It is free from pollution hazards and increase soil fertility
- ➢ Secrete growth promoting substances like IAA, IBA, NAA, aminoacids, proteins, vitamins, etc. They add sufficient amount of organic matter in soil
- ➢ The bioferilizers increase physico-chemical properties of soils such as soil structure, texture, water holding capacity, cation exchange capacity and pH by providing several nutrients and sufficient organic matter
- ➢ It fix nitrogen from air to plant

Azospirillum:

- ✈ *Azospirillum*, a free-living nitrogen-fixing bacteria closely associated with grasses
- ✈ Gram negative
- ✈ Motile bacteria
- ✈ Microaerophilic
- ✈ Non-fermentative
- ✈ Secretes some fungicides, enzymes but in minute amount
- ✈ Belonging to the order ***Rhodospirillales***

✈ **Associated with roots of monocots, including important crops, such as wheat, corn and rice**

Azospirillum	
Scientific classification	
Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Alphaproteobacteria
Order:	Rhodospirillales
Family:	Rhodospirillaceae
Genus:	***Azospirillum***
Type species	
Azospirillum lipoferum	
Species	
A. brasilense, *A. canadense*, *A. doebereinerae*, *A. fermentarium*, *A. formosense* *A. halopraeferens*, *A. humicireducens*, *A. irakense*, *A. largimobile*, *A. lipoferum* *A. melinis*, *A. oryzae*. *A. picis*, *A. rugosum*	

Isolation of *Azospirillum*

The roots are separated from the plants and thoroughly washed in running tap water

⬇

Then, they are transferred into 1 L flasks containing 0.5 L of sterile tap water and shaken for 30 minutes

⬇

The procedure is repeated three times, after which the same procedure is repeated with distilled water three times

⬇

The washed roots are moved to a sterile Petri dish and are cut into minuscule pieces with scissors pretreated with alcohol and burnt in the flame of an alcohol lamp

⬇

The root pieces are transferred into tubes containing 6mL of semi liquid selective media

⬇

The tubes are incubated at 30°C or at 37°C for 3-5 days

⬇

0.1 mL of culture liquid is transferred to the tubes containing fresh medium and incubated for 5-7 days

⬇

On semiliquid medium, azopirilla form a special subsurface growth ring

⬇

The tubes with special microaerophilic growth are inspected, and the microbial growth ring is observed under the microscope

↓

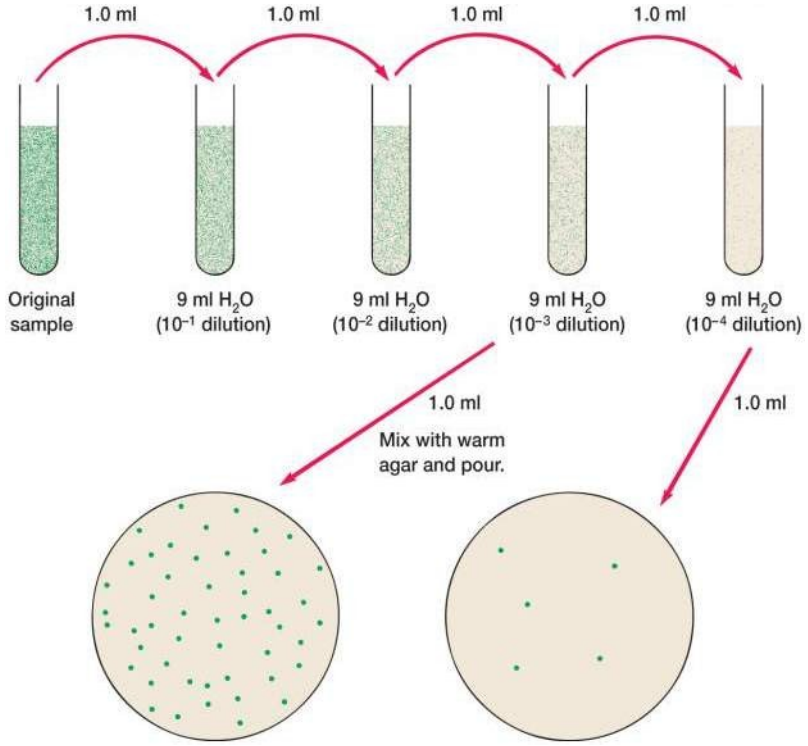
The samples with special helical movement are checked and selected

↓

Dilutions of the inoculum are plated on the same agar medium and incubated for 3-5 days

↓

The isolated colonies are selected



IDENTIFICATION:

- Preliminary identification is made with the immunodiffusion method
- The strains forming precipitation bands are selected
- Physiological and biochemical tests, immunochemical analysis, and 16S rRNA gene sequence analysis are used for the identification of isolated cultures

COLONY APPEARANCE:

CELLULAR	
Staining	Gram-negative to Gram-variable.
Morphology	Plump, slightly curved and straight rods, about 1.0 um in diameter and 2.1-3.8 um in length, often with pointed ends.
Motility	Motile in liquid media by a single polar flagellum. On solid media at 30`C numerous lateral flagella of shorter wavelength are also formed.
Specialized	Intracellular granules of poly-B-hydroxybutyrate present. Enlarged, pleomorphic forms

structures may occur in old, alkaline cultures or under conditions of excess oxygen
Division

COLONIAL

Solid surface: Colonies on potato agar are typically light or dark pink, often wrinkled and non-slimy.
Liquid : Turbidity

MASS CULTIVATION OF *Azospirillum:*

Sterilize the growth medium and inoculate with broth of mother culture prepared in advance

Incubate for 3-4 days at 30 - 32°C

↓

Test the cultures for its purity and transfer to a large fermenter, wait for 4-9 days for bacterial growth (for good bacterial growth make the device for its aeration)

↓

Allow to grow the bacteria either in a large fermenter containing broth or in small flasks as per demand

↓

Check the quality of broth

↓

Blend the broth with sterile carrier *e.g.* peat, lignite, farmyard manure and charcoal powder

↓

Pack the culture in polyethylene bags and keep at 25°C

↓

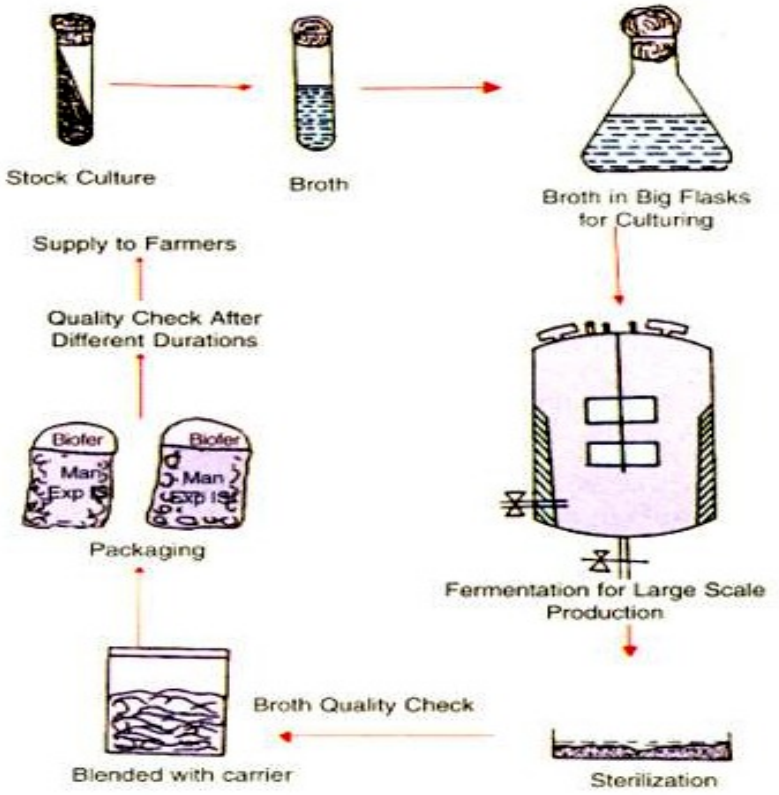
Check the quality of carrier culture

↓

Store at 4°C in a controlled-temperature room

↓

Supply to farmers



APPLICATION OF BIOFERTILIZERS

1. Seed treatment or seed inoculation
2. Seedling root dip
3. Main field application

Seed treatment

One packet of the inoculants is mixed with 200 ml of rice kanji to make a slurry. The seeds required for an acre are mixed in the slurry so as to have a uniform coating of the inoculants over the seeds and then shade dried for 30 minutes. The shade dried seeds should be sown within 24 hours. One packet of the inoculants (200 g) is sufficient to treat 10 kg of seeds.

Seedling root dip

This method is used for transplanted crops. Two packets of the inoculant is mixed in 40 litres of water. The root portion of the seedlings required for an acre is dipped in the mixture for 5 to 10 minutes and then transplanted.

Main field application

Four packets of the inoculant is mixed with 20 kgs of dried and powdered farm yard manure and then broadcasted in one acre of main field just before transplanting.

ACTINORHIZAL PLANTS:

Actinorhizal plants are a diverse group of woody species found on all continents (except Antarctica). Many are common plants, like alder, bayberry, sweet fern, etc. that one might pass every day. Others live in remote parts of the world. All play significant roles in the ecology of the soils in which they grow

MASS PRODUCTION *Frankia*

Inoculum preparation

- Prepare appropriate media for specific to the bacterial inoculant in 250 ml, 500 ml, 3 litre and 5 litre conical flasks and sterilize.
- The media in 250 ml flask is inoculated with efficient bacterial strain under aseptic condition
- Keep the flask under room temperature in rotary shaker (200 rpm) for 5- 7 days.
- Observe the flask for growth of the culture and estimate the population, which serves as the starter culture.
- Using the starter culture (at log phase) inoculate the larger flasks (500 ml, 3 litre and 5 litre) containing the media, after obtaining growth in each flask.
- The above media is prepared in large quantities in fermentor, sterilized well, cooled and kept it ready.
- The media in the fermentor is inoculated with the log phase culture grown in 5 litre flask. Usually 1 -2 % inoculum is sufficient, however inoculation is done up to 5% depending on the growth of the culture in the larger flasks.
- The cells are grown in fermentor by providing aeration (passing sterile air through compressor and sterilizing agents like glass wool, cotton wool, acid etc.) and given continuous stirring.
- The broth is checked for the population of inoculated organism and contamination if any at the growth period.
- The cells are harvested with the population load of 109 cells ml-1 after incubation period.
- There should not be any fungal or any other bacterial contamination at 10-6 dilution level

- It is not advisable to store the broth after fermentation for periods longer than 24 hours. Even at 4o C number of viable cells begins to decrease.

Processing of carrier material

The use of ideal carrier material is necessary in the production of good quality biofertilizer. Peat soil, lignite, vermiculite, charcoal, press mud, farmyard manure and soil mixture can be used as carrier materials. The neutralized peat soil/lignite are found to be better carrier materials for biofertilizer production The following points are to be considered in the selection of ideal carrier material.

- Cheaper in cost
- Should be locally available
- High organic matter content
- No toxic chemicals
- Water holding capacity of more than 50%
- Easy to process, friability and vulnerability.

Preparation of carrier material

- The carrier material (peat or lignite) is powdered to a fine powder so as to pass through 212 micron IS sieve.
- The pH of the carrier material is neutralized with the help of calcium carbonate (1:10 ratio) , since the peat soil / lignite are acidic in nature (pH of 4 - 5)
- The neutralized carrier material is sterilized in an autoclave to eliminate the contaminants.

Mixing the carrier and the broth culture and packing

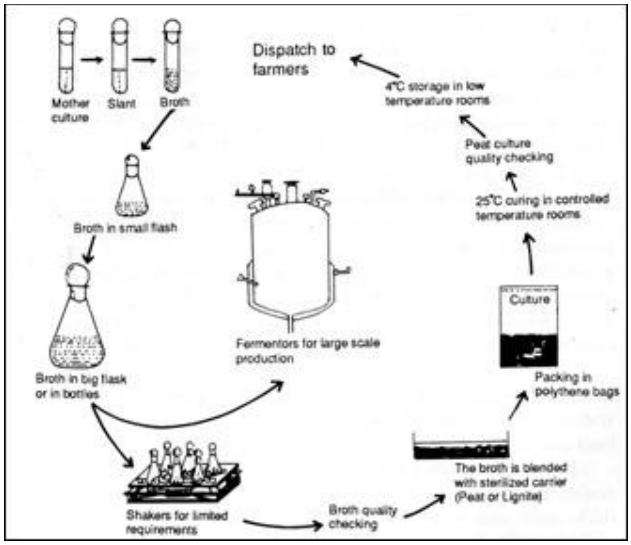
Inoculant packets are prepared by mixing the broth culture obtained from fermentor with sterile carrier material as described below:

Preparation of Inoculants packet

- The neutralized, sterilized carrier material is spread in a clean, dry, sterile metallic or plastic tray.

- The bacterial culture drawn from the fermentor is added to the sterilized carrier and mixed well by manual (by wearing sterile gloves) or by mechanical mixer. The culture suspension is to be added to a level of 40 – 50% water holding capacity depending upon the population.

- The inoculant packet of 200 g quantities in polythene bags, sealed with electric sealer and allowed for curing for 2 -3 days at room temperature (curing can be done by spreading the inoculant on a clean floor/polythene sheet/ by keeping in open shallow tubs/ trays with polythene covering for 2 -3 days at room temperature before packaging).



Schematic representation of mass production of bacterial biofertilizers

Specification of the polythene bags

- The polythene bags should be of low density grade.
- The thickness of the bag should be around 50 – 75 micron.

- Each packet should be marked with the name of the manufacturer, name of the product, strain number, the crop to which recommended, method of inoculation, date of manufacture, batch number, date of expiry, price, full address of the manufacturer and storage instructions etc.,

Storage of biofertilizerpacket

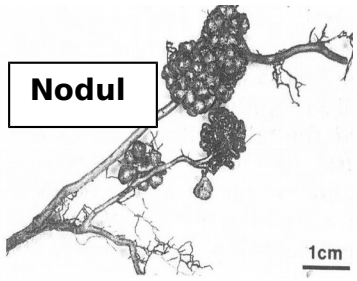
- The packet should be stored in a cool place away from the heat or direct sunlight.
- The packets may be stored at room temperature or in cold storage conditions in lots in plastic crates or polythene / gunny bags.

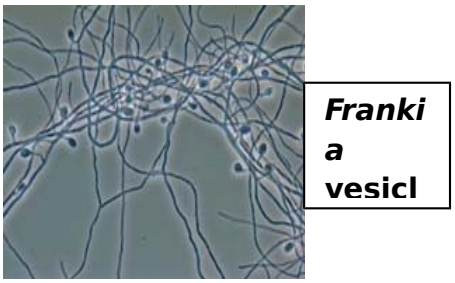
- The population of inoculant in the carrier inoculant packet may be determined at 15 days interval. There should be more than 109 cells / g of inoculant at the time of preparation and107 cells/ g on dry weight basis before expiry date.

NODULE APPEARANCE:

- ❖ Nodules have a range of colors from light yellow to orange, to various hues of brown and even red
- ❖ In size, they range from less than a millimeter for very young nodules up to more than ten centimeters for older nodules that have developed in loose sandy soil
- ❖ The larger nodules (more than 1 cm) generally only have active nitrogen-fixing tissue on the tip of young nodule lobes
- ❖ The older portions of the nodule in the interior of the cluster is frequently quite senescent and inactive
- ❖ Nodules are perennial and can continue to develop from season to season over several years

ACTINORRHIZAL NODULES APPEARANCE:

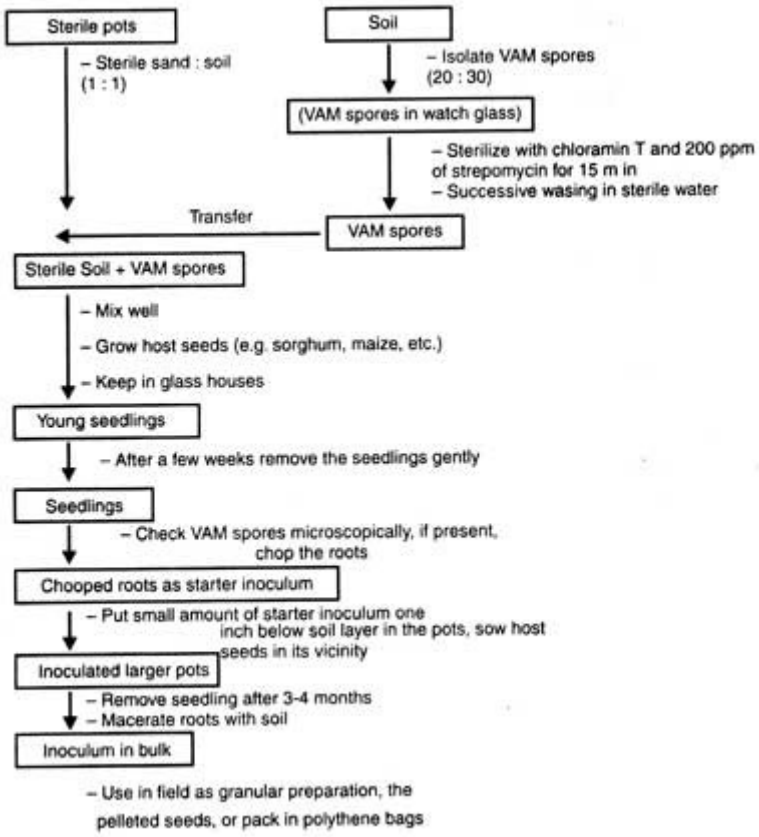




VAM

Vesicular arbuscular mycorrhizae (VA mycorrhizae, or **VAM**) are the most abundant of a group of symbiotic fungi that infect plant roots. ... These tubes originally grow from fungal spores, extending short distances (on the order of millimeters) into the soil in search of the roots of host plants

Mass Production of VAM:



Applications of Mycorrhizae :

- Increase nutrient uptake of plant from soil.
- P nutrition and other elements: N, K, Ca, Mg, Zn, Cu, S, B, Mo, Fe, Mn, Cl Increase diversity of plant.
- Produce uniform seedling. Significant role in nutrient recycling. More tolerant to adverse soil chemical constraints which limit crop production.
- Increase plant resistance to diseases and drought. Stimulate the growth of beneficial microorganisms. Improve soil structure.
- Stable soil aggregate – hyphal polysaccharides bind and aggregate soil particles.
- Increases absorption of phosphate by crops. uptake of zinc also increases.
- Increases uptake of water from soil. Increases uptake of sulphur from the soil
- Increases the concentration of cytokinins and chloroplast in plants.
- They protect plants during stress condition.

Biopesticide:

Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal applications and are considered **biopesticides**.

Classes of biopesticides

1. Biochemical pesticides:

- Naturally occurring substances that control pests by non-toxic mechanisms

- Includes substances that interfere with mating, such as insect sex pheromones, as well as various scented plant extracts that attract insect pests to traps

2. Microbial biopesticides:

- Consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient.

- Active ingredient is relatively specific for its target pest

- Eg: some Bt ingredients control moth larvae found on plants, other Bt ingredients are specific for larvae of flies and mosquitoes.

3. Plant – incorporated protectants (pips):

- Pesticidal substances that plants produce from genetic material that has been added to the plant.

- Eg: scientists can take the gene for the Bt pesticidal protein and introduce the gene into the plant's own genetic material.

- Then the plant, instead of the Bt bacterium, manufactures the substance that destroys the pest.

Advantages of biopesticides:

- Less toxic than conventional pesticides.
- Effect only the target pest and closely related organisms
- whereas conventional pesticides are broad spectrum pesticides.
- Effective in very small quantities and often decompose quickly, resulting in lower exposures and largely avoiding pollution problems caused by conventional pesticides.
- When used as a component of Integrated Pest Management programs, biopesticides can greatly reduce the use of conventional pesticides while crop yields remain high.

BACTERIAL BIOPESTICIDES:

- Mainly 4 categories:

1.Crystalliferous spore formers (Bacillus thuringiensis)

2.Obligate pathogens (Bacillus papiliae)

3.Potential pathogens (Serratia marcesens)

4.Facultative pathogens (Pseudomonas aeruginosa) Out of these four, 1 and 2 are important biopesticides.

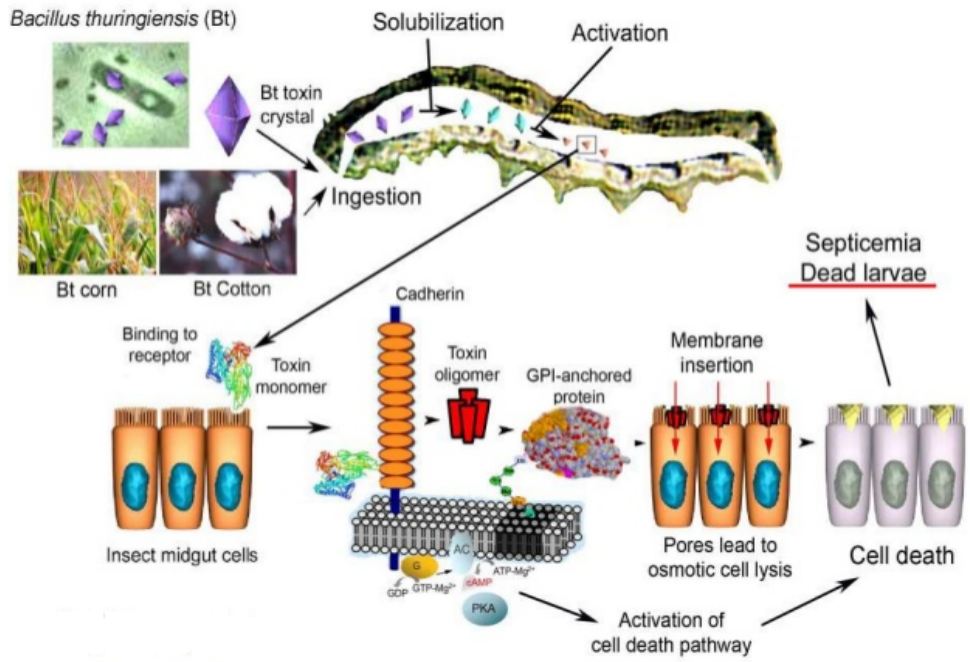
Bacillus thuringiensis

- Gram positive, spore forming, facultative bacterium with nearly 100 subspecies and varieties divided into 70 serotypes.
- Specific, safe and effective tool for insect control.

- Insecticidal property resides in Cry family of crystalline proteins that are produced in the parasporal crystals and are encoded by the cry genes

***B.thuringiensis* CRY proteins:**

- Cry proteins are globular molecules with 3 structural domain connected by single linkers.
- This 3 domain family is characterised by protoxins of two different lengths, one being longer with C – terminal extension necessary for toxicity.
- This extension also has a characteristic role in crystal formation within the bacterium.
- Cry proteins are responsible for feeding cessation and death of the insect.



Mechanism of Cry Protein:

- Cry protoxins are ingested and then solubilised, releasing a protease resistant biologically active endotoxin, before it is being digested by protease of the gut to remove amino acids from its C and N terminal ends.
- The C terminal domain of the active toxin binds to the specific receptors on brush border membranes of the midgut.
- It is followed by the insertion of the hydrophobic region of the toxin into the cell membrane
- This creates a disruption in the osmotic balance because of the formation of transmembrane pores.
- Ultimately cell lysis occurs in the gut wall leading to leakage of gut contents.
- This induces starvation and lethal septicaemia of the target pest.

Bioplastics:

Bioplastics can be of many types and are obtained from various biological sources including plants and microbes. This article focuses on one such material, **polyhydroxyalkanoates**-commonly known as **PHAs**- and their microbial biosynthesis.

Biopolastics producing Microbes:

Many living organisms, mainly plants and bacteria produce PHAs. However, microorganisms are more suitable for the industrial production of PHAs due to the fact that plant cells can only accumulate low yields of PHAs i.e. less than 10% (w/w) without adversely affecting their growth. In contrast, bacteria are known to store up PHAs at levels as high as 90% (w/w) of their dry cell weight.

A wide range of Gram-positive and Gram-negative bacteria including *Aeromonas hydrophila, Alcaligenes latus, Pseudomonas* sp, *Bacillus* sp, and *Methylobacterium* sp naturally synthesise PHAs. These bacteria produce PHA as a carbon and energy storage compounds under imbalanced nutritional conditions. When carbon is in excess and the other nutrients such as nitrogen or phosphorous or oxygen is limited, PHA is accumulated in the form of water-insoluble granules in their cytoplasms.

Biosynthesis

Biosynthesis PHA, a culture of a micro-organism such as *Cupriavidus necator* is placed in a suitable medium and fed appropriate nutrients so that it multiplies rapidly.

⇓

Once the population has reached a substantial level, the nutrient composition is changed to force the micro-organism to synthesize PHA.

⇓

The yield of PHA obtained from the intracellular granule inclusions can be as high as 80% of the organism's dry weight.

⇓

The biosynthesis of PHA is usually caused by certain deficiency conditions (e.g. lack of macro elements such as phosphorus, nitrogen, trace elements, or lack of oxygen) and the excess supply of carbon sources

⇓

Polyesters are deposited in the form of highly refractive granules in the cells.

Depending upon the microorganism and the cultivation conditions, homo- or copolyesters with different hydroxyalkanic acids are generated.

⇓

PHA granules are then recovered by disrupting the cells. Recombinant *Bacillus subtilis* str. pBE2C1 and *Bacillus subtilis* str. pBE2C1AB were used in production of polyhydroxyalkanoates (PHA) and it was shown that they could use malt waste as carbon source for lower cost of PHA production.

⇩

PHA synthases are the key enzymes of PHA biosynthesis. They use the coenzyme A - thioester of (r)-hydroxy fatty acids as substrates.

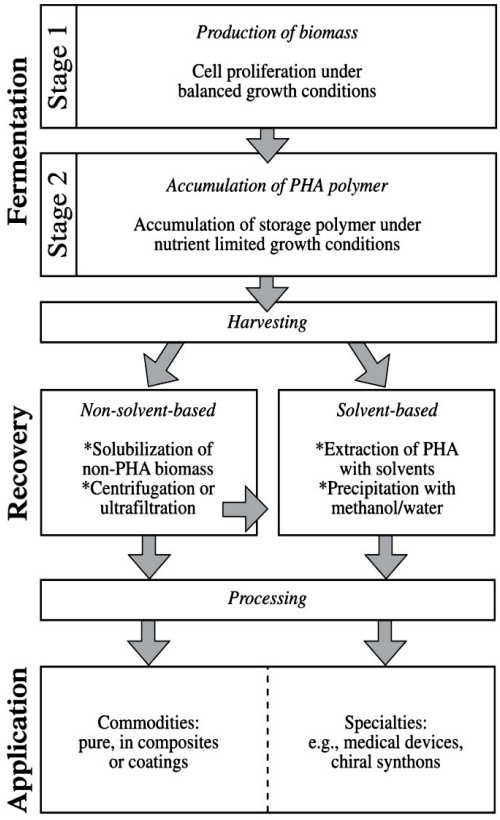
⇩

The two classes of PHA synthases differ in the specific use of hydroxy fatty acids of short or medium chain length.

⇩

The resulting PHA is of the two types:

- Poly (HA SCL) from hydroxy fatty acids with short chain lengths including three to five carbon atoms are synthesized by numerous bacteria, including *Cupriavidus necator* and *Alcaligenes latus* (PHB).
- Poly (HA MCL) from hydroxy fatty acids with medium chain lengths including six to 14 carbon atoms, can be made for example, by *Pseudomonas putida*.



Applications:

Polyhydroxyalkanoates have a great variety of characteristics, and their sustainability, biodegradability, and biocompatibility mean that many industries would benefit from utilizing them further. Here are some applications in which PHAs have become very useful:

- Single use packaging for foods, beverages, consumer products, etc
- Medical applications like sutures, bone marrow scaffolds, bone plates, etc
- Agricultural foils and films

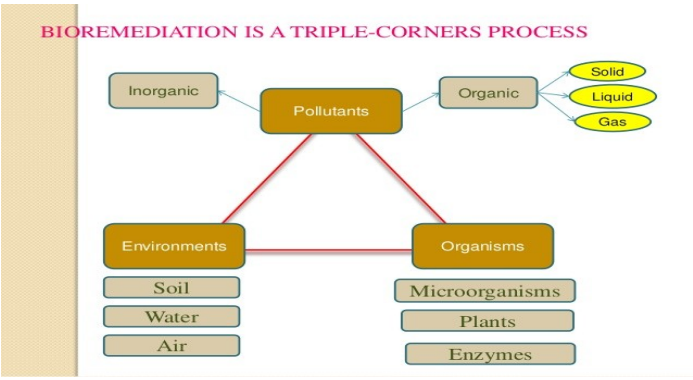
Bioremediation

Definition:

- Bioremediation refers to the process of using microorganisms to remove the environmental pollutants or prevent pollution.
- The removal of organic wastes by microbes for environmental clean-up is the essence of bioremediation.
- The other names used for bioremediation are biotreatment, bioreclamation and biorestoration.
- Xenobiotics broadly refer to the unnatural, foreign and synthetic chemicals such as pesticide, herbicide & other organic compounds.

Types of bioremediation:

1. Biostimulation
2. Bioaugmentation
3. Intrinsic bioremediation.



Use of Microbes in Bioremediatrion:

Use of bacteria in bioremediation:

- Greatly affected by unstable climatic and environmental factors from moisture to temperature.
- For examples, pH in soil is slightly acidic; petroleum hydrocarbon degrading bacteria do not work well < 10º C.
- These microbes are usually thermophilic anaerobic.
- Fertilizers are needed. Seeding or bioaugmentation could be useful too.
- They contain monooxygenases and dehydrogenases to break down organic matters including most toxic substances.

Pseudomonas: Genetically engineered bacteria *(Pseudomonas)* with plasmid producing enzymes to degrade octane and many different organic compounds from crude oil.

A selected list of genetically engineered microorganisms:

GEMs	XENOBIOTICS
Pseudomonas putida	Mono-and dichloro aromatic compounds
P.diminuta	Parathion
P.oleovorans	Alkane
P.cepacia	2,4,5-Trichlorophenol
Acinetobacter species	4-Chlorobenzene

Alcaligenes species	2,4-Dichlorophenoxy acetic acid

Use of fungi in bioremediation:

- Candida can degrade formaldehyde. Gibeberella can degrade cyanide.
- Slurry-phase bioremediation is useful too but only for small amounts of contaminated soil.
- Composting can be used to degrade household wastes.

White rot fungi:

- White rot fungi can degrade organic pollutants in soil and effluent and decolorize kraft black liquor, e.g. ***Phanerochaete chrysosporium*** can produce aromatic mixtures with its lignolytic system.
- Pentachlorophenol, dichlorodiphenyltrichloroethane (e.g. DDT), even TNT (trinitrotoluene) can be degraded by white rot fungi.

Advantages of bioremediation:

- Bioremediation is a natural process and is therefore perceived by the public.
- Bioremediation is useful for the complete destruction of a wide variety of contaminants.
- Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible.
- Bioremediation can often be carried out on site, often without causing a major disruption of normal activities.
- Bioremediation can prove less expensive than other technologies that are used for cleanup of hazardous waste.

Disadvantages of bioremediation:

- Bioremediation is limited to those compounds that are biodegradable.
- Not all compounds are susceptible to rapid and complete degradation.
- There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound.

- Biological processes are often highly specific. Microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.
- It is difficult to extrapolate (deduce) from bench and pilot-scale studies to fullscale field operations.
- Bioremediation often takes longer than other treatment options.

Xebobiotics:

• It is derived from a greek word "XENOS" meaning 'foreign or strange

• Xenobiotics are those chemicals which are man-made and do not occur naturally in nature.

• They are usually synthesized for industrial or agricultural purposes e.g. aromatics, pesticides, hydrocarbons, plastics , lignin etc.

• They are also called RECALCITRANTS as they can resist degradation to maximum level.

Biodegradation:

• The term biodegradation is "Breakdown of a substance catalyzed by enzymes in vitro or in vivo.

• In other words, defined as the ability of microorganisms to convert toxic chemicals (xenobiotics) to simpler non-toxic compounds by synthesis of certain enzymes

• Biodegradation of xenobiotics can be affected by substrate specificity, nutrition source, temperature, pH etc.

Sources of xenobiotics:

1. Petrochemical industry: -oil/gas industry, refineries. - produces basic chemicals e.g. vinyl chloride and benzene

2. Plastic industry: - closely related to the petrochemical industry - uses a number of complex organic compounds -such as anti-oxidants, plasticizers, cross-linking agents

3. Pesticide industry: - most commonly found. -structures are benzene and benzene derivatives,

4. Paint industry: - major ingredient are solvents, - xylene, toluene, methyl ethyl ketone, methyl

5. Others: - Electronic industry, Textile industry, Pulp and Paper industry, Cosmetics and Pharmaceutical industry, Wood preservation

BIODEGRADATION OF PESTICIDES:

• Pesticides are substances meant for destroying or mitigating any pest.

• They are a class of biocide.

• The most common use of pesticides is as plant protection products (also known as crop protection products).

• It includes: herbicide, insecticide, nematicide, termiticide, molluscicide, piscicide, avicide, rodenticide, insect repellent, animal repellent, antimicrobial, fungicide, disinfectant, and sanitizer.

Different methods: -

a) Detoxification: Conversion of the pesticide molecule to a non-toxic compound. A single moiety in the side chain of a complex molecule is disturbed(removed), rendering the chemical non-toxic.

b) Degradation: Breakdown or transformation of a complex substrate into simpler products leading to mineralization. E.g. Thirum (fungicide) is degraded by a strain of Pseudomonas and the degradation products are dimethylamine, proteins, sulpholipids, etc

c) Conjugation (complex formation or addition reaction): An organism makes the substrate more complex or combines the pesticide with cell metabolites. Conjugation or the formation of addition product is accomplished by those organisms catalyzing the reaction of addition of an amino acid, organic acid or methyl crown to the substrate thereby inactivating the pestcides

d) Changing the spectrum of toxicity: Some pesticides are designed to control one particular group of pests, but are metabolized to yield products inhibitory to entirely dissimilar groups of organisms, for e.g. the fungicide PCNB is converted in soil to chlorinated benzoic acids that kill plants.

biodegradation of plastics:

• Plastic is a broad name given to different polymers with high molecular weight, which can be degraded by various processes.

• The biodegradation of plastics by microorganisms and enzymes seems to be the most effective process.

• It consist of two steps- fragmentation and mineralization. But at the core, reaction occurring at molecular level is oxidation and hydrolysis.

• The decomposition of major condensation polymers (e.g. polyesters and polyamides) takes place through hydrolysis, while decomposition of polymers in which the main chain contains only carbon atoms (e.g. polyvinyl alcohol, lignin) includes oxidation which can be followed by hydrolysis of the products of oxidation.

Method of degradation of plastics:

- **Hydrolysis-** The process of breaking these chains and dissolving the polymers into smaller fragments is called hydrolysis. E.g. Pseudomonas sps Polymeric Chains is broken down into constituent parts for the energy potential by microorganisms. Monomers are readily available to other bacteria and is used. Acetate and hydrogen produced is used directly by methanogens. Other molecules, such as volatile fatty acids (VFAs) with a chain length greater than that of acetate is first catabolized into compounds that can be directly used by methanogens.
- **Acidogenesis-** This results in further breakdown of the remaining components by acidogenic (fermentative) bacteria into ammonia, ethanol, carbon dioxide, and hydrogen sulfide. E.g Streptococcus acidophilus.

- **Acetogenesis**- Simple molecules created through the acidogenesis phase are further digested by Acetogens to produce largely acetic acid, as well as carbon dioxide and hydrogen.

- **Methanogenesis-** Here, methanogens use the intermediate products of the preceding stages and convert them into methane, carbon dioxide, and water. These components make up the majority of the biogas emitted. Methanogenesis is sensitive to both high and low pHs and occurs between pH 6.5 and pH 8. The remaining, indigestible material the microbes cannot use and any dead bacterial remains constitute the digestate.

UNIT III:

Single cell protein:

- The term "**Single Cell Protein**" refers to the total **protein** extracted from the pure cultures of microorganisms (e.g. yeast, algae, filamentous fungi, bacteria) and can be used as a **protein**-rich food supplements by humans and animals.
- Used to named as Microbial Proteins.

Microalgae:

Microalgae have been used as **food** by humans for thousands of years. **Microalgae** can convert solar energy to chemical energy by fixing CO_2, and its efficiency is ten times greater than terrestrial plants.

1. **Single Cell Protein:**
   - SCP are dried cells of micro organisms which can be used as dietary protein supplement.
   - They are used as animal feed & can be used for human feed as protein supplement.
   - Also called 'Novel Food' & 'Minifood'.
2. **Raw materials**

   • Production of SCP requires micro-organisms that serve as the protein source and the substrate that is biomass on which they grow

• There is a variety of both the sources that can be used for the production of SCP.

• The biomass used can be plant biomass or organic biomass.

• The micro-organisms used belong to the group of Algae, Fungi and Bacteria.

3. **A list of the micro-organisms used for SCP production**:

   ✓ **Fungi•** *Aspergillus fumigatus• Aspergillus niger• Rhizopus cyclopium*

   ✓ **Yeast•** *Saccharomyces cerevisae• Candida tropicalis• Candida utilis*

   ✓ **Algae•** *Spirulina sps.• Chlorella pyrenoidosa• Chondrus crispus*

   ✓ **Bacteria•** *Pseudomonas fluroescens• Lactobacillus• Bacillus megaterium*

4. **Biomass:**

• Biomass also plays a very important role in the production of SCP.

• Selection of biomass depends on the micro-organisms used for the production.

• For eg. Algae are cultivated on sewage whereas Yeast are cultured on agro-industrial wastes.

✓ **Algal Biomass:**

• Algae grows auto- tropically.

• Requires low intensity of light.

• Temperature – 35 - 40 C & pH – 8. 5 -10.5

• Cultivated in large trenches of sewage oxidation ponds.

✓ **Bacterial & Fungal biomass:**

• Bacteria & fungi can be grown easily on a wide range of substrates

• They require a minimum temperature of 15º-34ºc & a pH of 5- 7.

✓ **Yeast biomass**:

• Cultivated on agro- industrial wastes such as molasses, starchy materials, fruit pulp, wood pulp, etc.

• Requires a temperature of 30 -34 c & pH of 3.5- 4.5.

• Also requires addition of inorganic acids & sulphur supplements in the form of salts.

5. **SCP production:**

• Selection of suitable strain

• Fermentation

• Harvesting

• Post harvest treatment

• SCP processing for food

✈ **Selection of strain:**

• It a very critical step as the quality of protein depends totally on the microbe that is used for the production.

• Thus careful selection of the strain should be done.

• Care should be taken that the selected strain should not produce any toxic or undesirable effects in the consumer.

✈ **Fermentation:**

• It can be carried out in the fermentor which is equipped with aerator, thermostat, pH, etc. or in the trenches or ponds.

• Microbes are cultured in fed- batch culture.

• Engineers have developed deep lift fermentor & air lift fermentor .

✈ **Harvesting:**

• When the colonies of microbes are fully developed, they are then harvested.

• The bulk of cells are removed from the fermentor by decantation.

✈ **Post harvest treatment:**

• After harvesting, the cells are subjected to a variety of processes.

• Post harvesting treatments includes steps like separation by centrifugation, washing, drying, etc.

6. Processing for food:

It includes

1. Liberation of cell proteins by destruction of indigestible cell wall.

A. Mechanical methods: • Crushing, crumbling, grinding, pressure homogenization, etc.

B. Chemical methods: • Enzymes & salts are used to digest or disrupt the cell wall.

• Salts like NaCl, sodium dodecyl sulfate, etc. whereas nuclease enzymes are used.

C. Physical methods: • Freeze- thaw, osmotic shock, heating & drying.

2. Reduction of nucleic acid content:

• Chemical & enzymatic treatments are preferred.

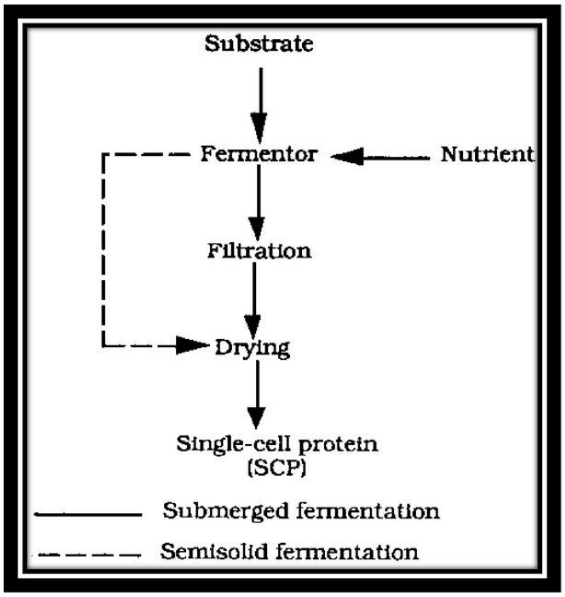
• Chemicals which are used includes acidified alcohol, salts, acids & alkalies.

• Use of such chemicals leads to formation of lygino-alanine which causes hypersensitivity skin reactions.

• Enzymes which are used include ribonuclease & nuclease enzymes

• These enzymes can be used exogenously or can be induced endogenously.

Basic Steps of SCP production:



7. **Applications**:

    1. **As protein supplemented food**
        - Also source of vitamins, amino acids, minerals, crude fibers, etc.
        - Supplemented food for undernourished children.
    2. **As health food**
        - Controls obesity
        - Provides instant energy
        - Example- Spirulina- part of diet of US Olympic team.
    3. **In therapeutic and natural medicines**
        - Reduce body weight, cholesterol, stress.
        - Lowers blood sugar level in diabetic(due to presence of B - linolenic acid)
        - Prevents accumulation of cholesterol in body.
        - Healthy eyes and skin (beta carotene)
        - Beta carotene (anti cancer substance- UN National Cancer Research Institute)
        - Increase lactation.
    4. **In cosmetics**
        - Important role in maintaining healthy hair (vitamin A and B).
        - Many herbal beauty products.
        - Biolipstics and herbal face cream (Phycocyanin).
        - Capable of replacing coal tar dye based cosmetics.
    5. **Poultry and cattle feed**

• Excellent, convenient source of protein and other nutrients.
• Used to feed cattle, fishes etc.

Algae as a Source of Pharmaceuticals :

- Algae are a rich and varied source of pharmacologically active natural products and nutraceuticals. While nutraceutical and pharmaceutical content in the baseline algae strain is very small, current market values for these products are extremely high.
- The major products currently being commercialized or under consideration for commercial extraction include carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, sterols, and biologically active molecules for use in human and animal health.
- The upcoming sections will bring into focus the use of algae as a potential source of pharmaceutical and nutraceutical ingredients.

Algae as a Source of Pharmaceuticals

Use of algae, especially the cyanobacteria (blue-green algae), for antibiotics and pharmacologically active compounds has received ever increasing interest. There are a range of pharmaceutical products derived from algae. Some of them include:

- – Antimicrobials, Antivirals & Antifungals
- -Neuroprotective Products
- -Therapeutic proteins
- -Drugs

1. Antimicrobials, Antivirals & Antifungals

Both microalgae and macroalgae exhibit antimicrobial activity which finds use in various pharmaceutical industries.

Role of Microalgae:

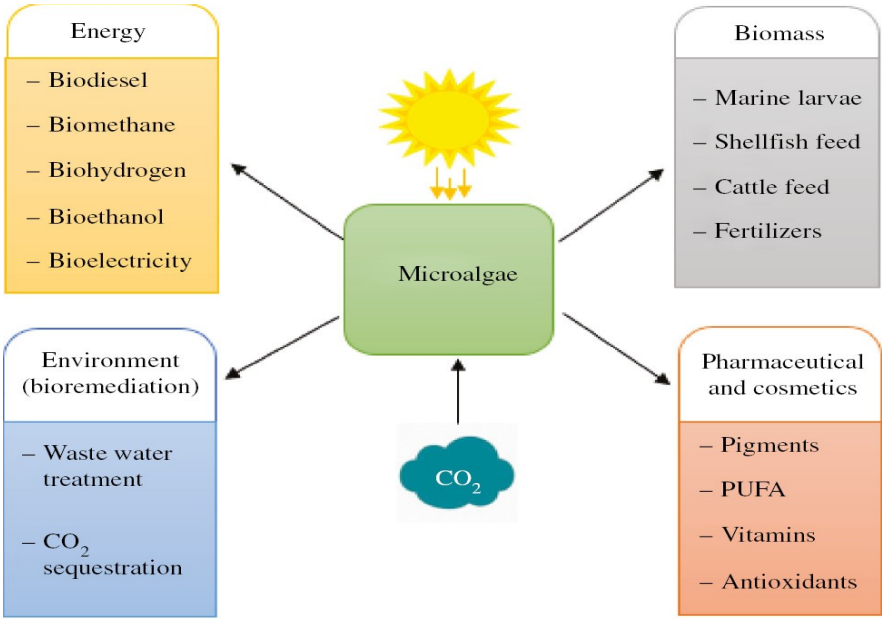
- * Microalgae, such as *Ochromonas sp., Prymnesium* and a number of blue green algae produce toxins that may have potential pharmaceutical applications
- * Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial, antifungal and antiviral activity
- * The biological activities of the algae may be attributed to the presence of volatile compounds, some phenols, free fatty acids and their oxidized derivatives

Role of macroalgae:

- * There are numerous reports of macroalgae derived compounds that have a broad range of biological activities, such as antibiotic, antiviral, anti-neoplastic, antifouling, anti-inflammatory, cytotoxic and antimitotic
- * In the past few decades, macroalgae have been widely recognised as producers of a broad range of bioactive metabolites
- * Such antimicrobial properties enable macroalgae to be used as natural preservatives in the cosmetic industry.
- * The highest percentage of antimicrobial activity was found in Phaeophyceae (84%), followed by Rhodophyceae (67%) and Chlorophyceae (44%).
- * Red and brown macroalgae extracts show significant potential as anti-pathogenic agents for use in fish aquaculture.

Fuel Production:

Microalgae has been reported to **produce** biogas as source of **fuel**, although the yield of biogas formation is quite low because of the sensitivity of **algal** cells to bacterial degradation and low carbon and nitrogen (C:N) ratio, which leads to the formation of inhibitor (ammonia).



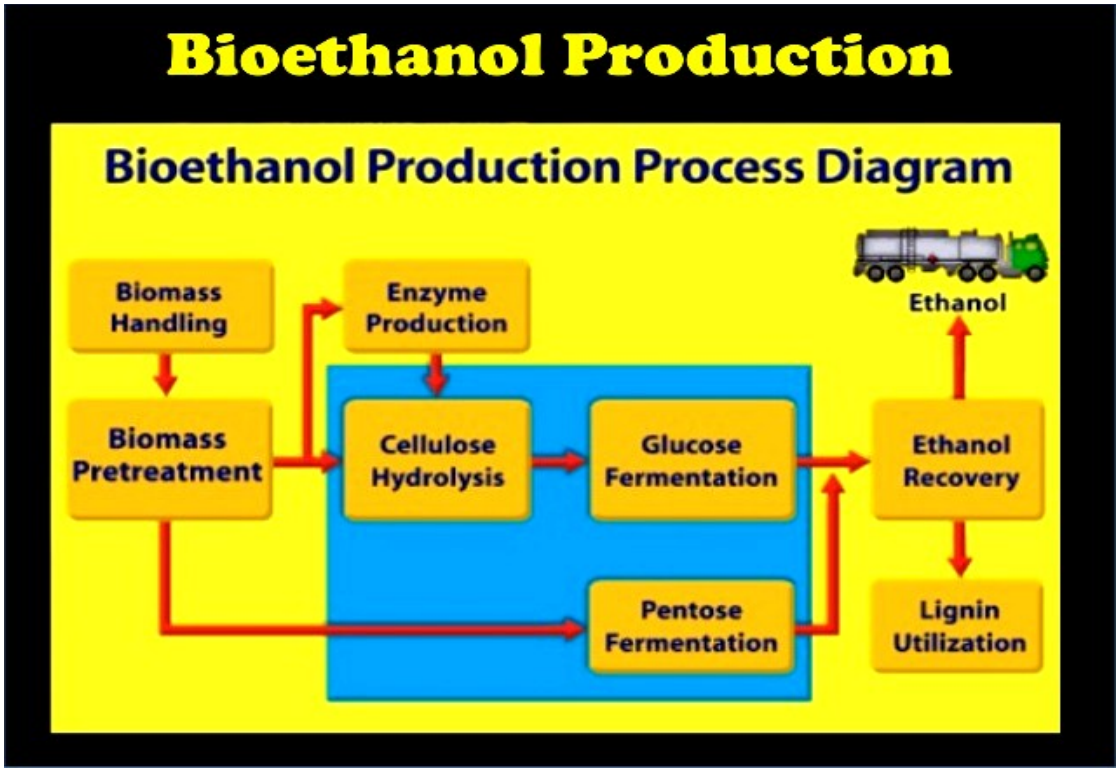
Biofuels are liquid fuels that have been developed from other materials such as plants or animal waste matter by microbial action

Bioethanol:

- Bioethanol is an alcohol made by fermentation, mostly from carbohydrates produces in sugar or starch crops such as corn or sugarcane.
- Cellulosic biomass, derived from non-food sources such as trees and grasses, is also being developed as a feedstock for ethanol production.
- Used to substitute petrol fuel for the road transport vehicles
- One of the widely used alternative automotive fuels in the world (Brazil & USA are the largest ethanol producers)
- Much more environment friendly and have low toxicity level

Raw Source:

- First generation biofuels are made from **the sugars and vegetable oils** found in arable crops, second generation biofuels are made from **lignocellulosic biomass or woody crops, agricultural residues or waste plant material** (from food crops that have already fulfilled their food purpose)
- The feedstock used to generate second-generation biofuels thus either grows on arable lands, but are just byproducts of the actual harvest (main crop) or they are grown on lands which cannot be used to effectively grow food crops

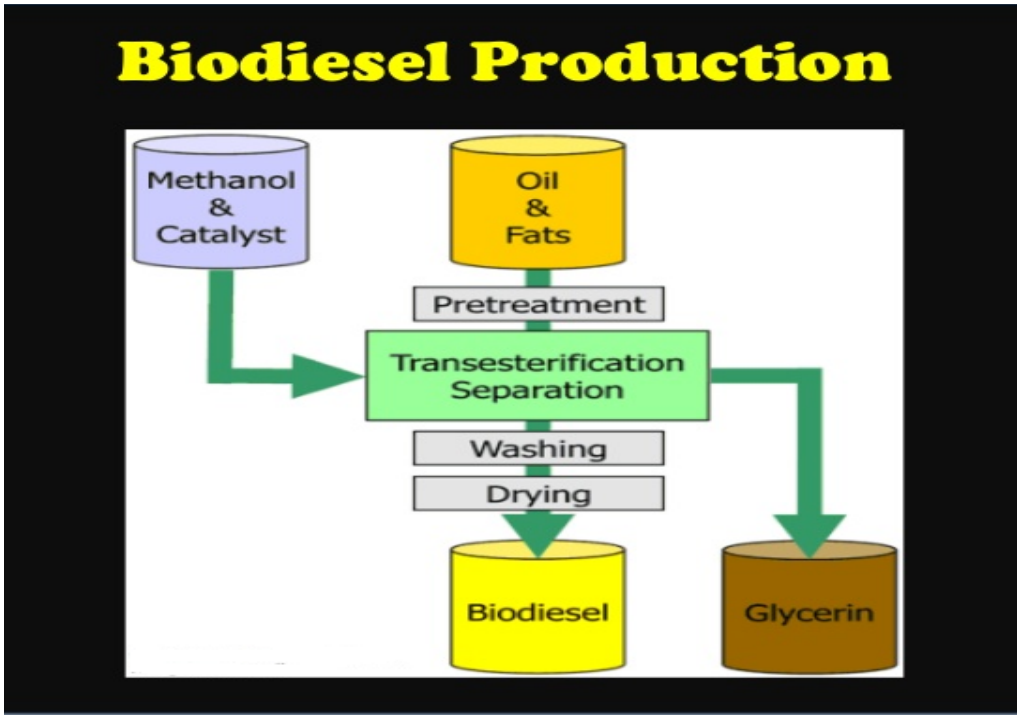


Applications of Bioethanol:

- Transport fuel to replace gasoline Fuel for power generation by thermal combustion
- Fuel for fuel cells by thermochemical reaction
- Fuels in cogeneration systems
- Feedstock in the chemical industry
- Blending ethanol with small portion of gasoline is more cost-effective
-

Biodiesel:

- Biodiesel is a variety of ester-based oxygenated fuels derived from natural, renewable biological sources such as vegetable oils.
- Biodiesel operates in compression ignition engines like petroleum diesel thereby requiring no essential engine modifications.
- Unlike fossil diesel, pure biodiesel is biodegradable, non- toxic and essentially free of sulphur and aromatics.



Uses of Biodiesel:

- Biodiesel is environmentfriendly.
- It can help reduce dependency on foreign oil.
- It helps to lubricate the engine itself, decreasing engine wear.
- It can be used in almost any diesel with little or no engine modification.
- It is safer than conventional diesel.
- Less global warming.

Uses of biofuels:

1. **Cars and Trucks:** Diesel cars and trucks can run on biodiesel.
2. **Aircraft:** Recent testing has shown the viability of biofuel use in the aviation industry, and use of biofuels to power aircraft is expected to increase substantially in the next decade.
3. **Off-Road Equipment**: A large percentage of off-road equipment -- such as vehicles used in agriculture, mining, forestry, construction, and power and heat production -use diesel fuel, making this equipment suitable for biodiesel use
4. **Small Engines:** Small engines, like those found in lawn mowers and chainsaws, can use ethanol blends up to 10 percent without problems

Advantages of biofuels:

1. **Cost:** Biofuels have the potential to be significantly less expensive than gasoline and other fossil fuels.
2. **Source material:** Whereas oil is a limited resource that comes from specific materials, biofuels can be manufactured from a wide range of materials including crop waste, manure, and other byproducts. This makes it an efficient step in recycling.
3. **Renewability:** It takes a very long time for fossil fuels to be produced, but biofuels are much more easily renewable as new crops are grown and waste material is collected.
4. **Security:** Biofuels can be produced locally, which decreases the nation's dependence upon foreign energy
5. **Economic stimulation:** Because biofuels are produced locally, biofuel manufacturing plants can employ hundreds or thousands of workers, creating new jobs in rural areas.
6. **Lower carbon emissions:** When biofuels are burned, they produce significantly less carbon output and fewer toxins, making them a safer alternative to preserve atmospheric quality and lower air pollution.

Disadvantages of biofuels:

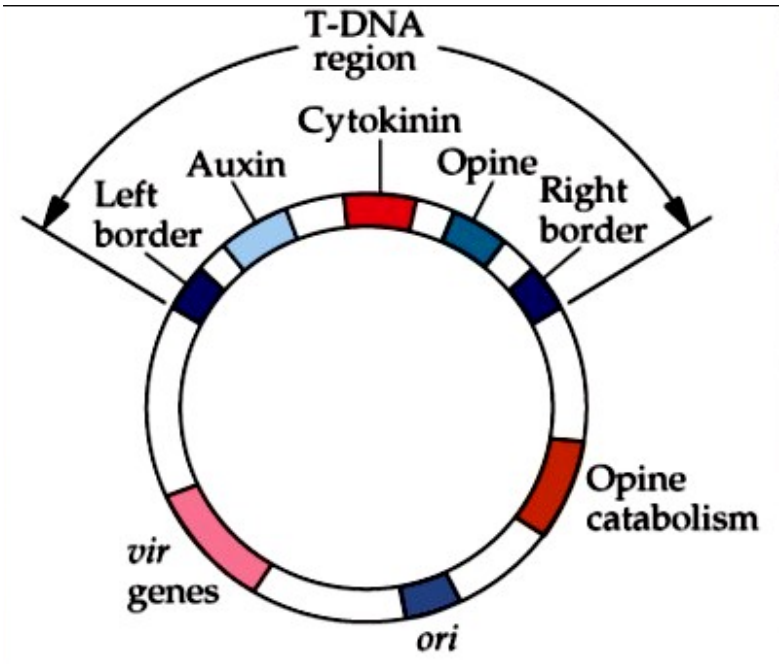
1. **Production carbon emissions:** Several studies have been conducted to analyze the carbon footprint of biofuels, and while they may be cleaner to burn, there are strong indications that the process to produce the fuel including the machinery necessary to cultivate the crops and the plants to produce the fuel - has hefty carbon emissions.
2. **High cost:** To refine biofuels to more efficient energy outputs, and to build the necessary manufacturing plants to increase biofuel quantities, a high initial investment is often required.
3. **Food prices:** As demand for food crops such as corn grows for biofuel production, it could also raise prices for necessary staple food crops.
4. **Food shortages:** There is concern that using valuable cropland to grow fuel crops could have an impact on the cost of food and could possibly lead to food shortages.
5. **Water use:** Massive quantities of water are required for proper irrigation of biofuel crops as well as to manufacture the fuel, which could strain local and regional water resources.

UNIT IV:

Ti plasmid

- A **Ti** or **tumour inducing** plasmid is a plasmid that often, but not always, is a part of the genetic equipment that *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* use to transduce their genetic material to plants.
- The Ti plasmid is lost when *Agrobacterium* is grown above 28 °C. Such cured bacteria do not induce crown galls, i.e. they become avirulent.
- pTi and pRi share little sequence homology but are functionally rather similar.
- The Ti plasmids are classified into different types based on the type of opine produced by their genes.
- The different opines specified by pTi are octopine, nopaline, succinamopine and leucinopine.
- The plasmid has 196 genes that code for 195 proteins. There is one structural RNA.
- The plasmid is 206,479 nucleotides long, the GC content is 56% and 81% of the material is coding genes. There are no pseudogenes.
- The modification of this plasmid is very important in the creation of transgenic plants.

Structure of Ti Plasmid:



Vir (virulent) Gene:

1. Transfer the T-DNA to plant cell

2. Acetosyringone (AS) (a flavonoid) released by wounded plant cells activates vir genes.

3. virA,B,C,D,E,F,G (7 complementation groups, but some have multiple ORFs), span about 30 kb of Ti plasmid. **Function of vir genes:**

- ✓ **virA** - transports acetosyringone into bacterium, activates virG post-translationally (by phosphorylation)
- ✓ **virG** - promotes transcription of other vir genes
- ✓ **virD2-** endonuclease/integrase that cuts T-DNA at the borders but only on one strand.
- ✓ **virE2 -** can form channels in membranes
- ✓ **virE1 -** chaperone for virE2
- ✓ **virD2** & **virE2** also have NLSs, gets T-DNA to the nucleus of plant cell
- ✓ virB - operon of 11 proteins, gets T-DNA through bacterial membranes

Opines:

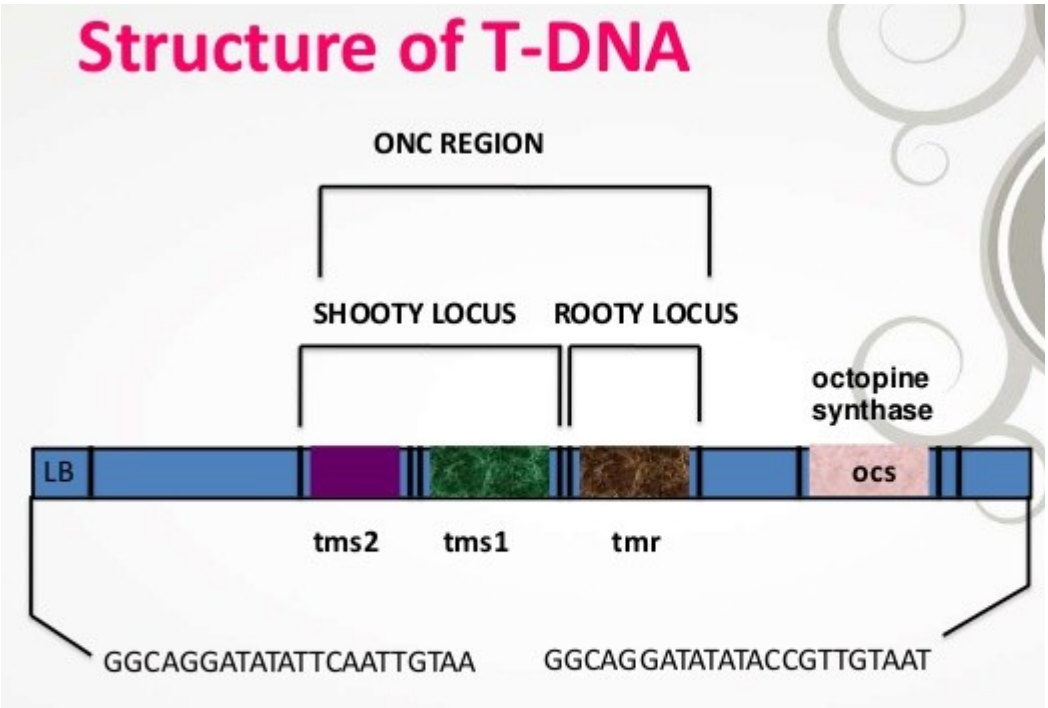
- ❖ Derivatives of amino acids synthesized by T-DNA
- ❖ Ti plasmids can be classified according to the opines produced:

  1. Nopaline plasmids

  2. Octopine plasmids

  3. Agropine plasmids

- ❖ Nopaline plasmids : carry gene for synthesizing nopaline in the plant and for utilization (catabolism) in the bacteria.
- ❖ Octopine plasmids : carry genes to synthesize octopine in the plant and catabolism in the bacteria.
- ❖ Agropine plasmids : carry genes for agropine synthesis and catabolism.



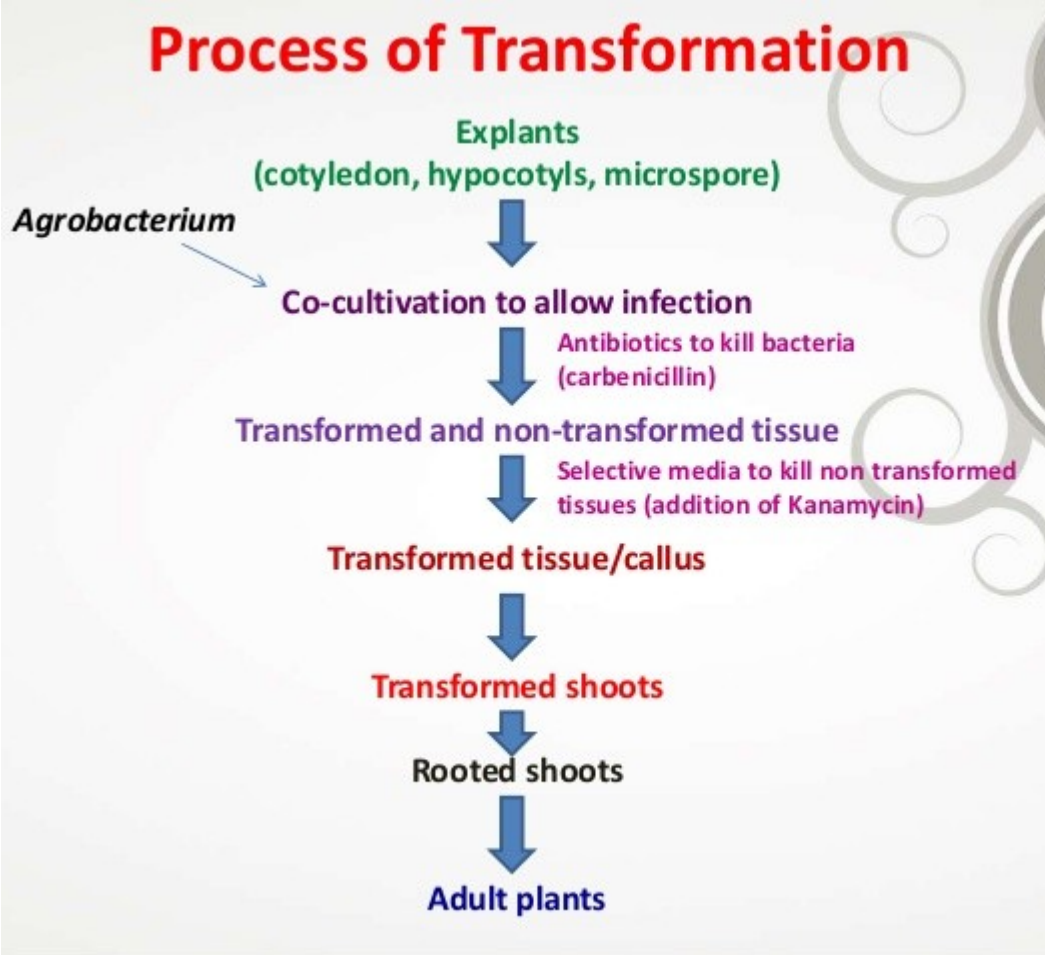
Ti Plasmid-Derived Vector Systems:

- ❖ Using Ti plasmid as a vector it is possible to insert a desiredϖ DNA sequence (gene) into the T DNA region of Ti plasmid.
- ❖ There are several limitations to use Ti plasmids directly as cloning vectors :-
  - LARGE SIZE
  - TUMOR INDUCTION PROPERTY
  - ABSENCE OF UNIQUE RESTRICTION SITES
- ❖ Agrobacterium plasmids are disarmed by deleting naturally occurring T-DNA encoded oncogenes and replacing them with foreign genes of interest.
  - The right and left border sequences of T-DNA which is required for T-DNA integration.
  - A multiple cloning site.
  - An origin of replication
  - A selectable marker gene

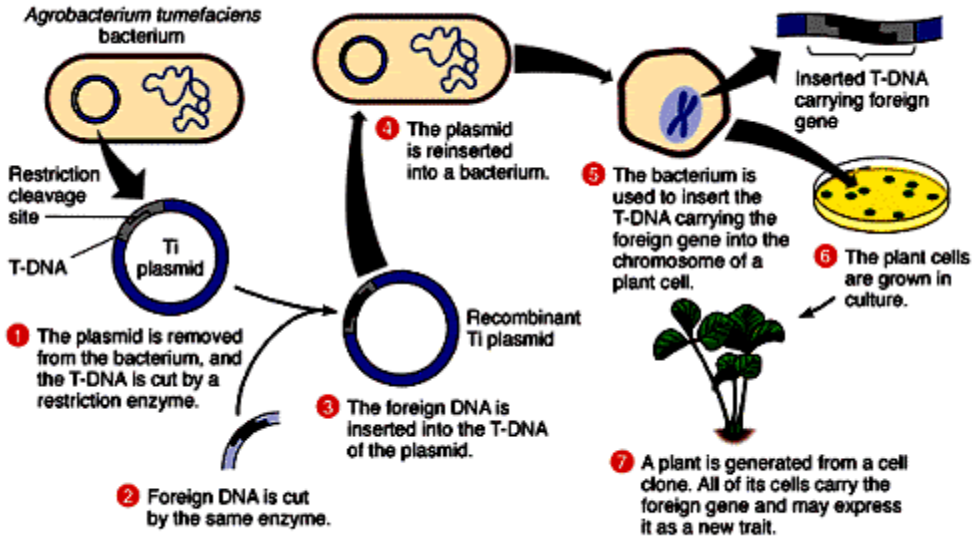
Agrobacterium mediated transformation:

The important requirements for Agrobacterium- mediated gene transfer in higher plants are as follows:-

- ➢ The plant explants must produce acetosyringone for activation of Vir genes.
- ➢ The induced Agrobacterium should have access to cells that are competent for transformation.
- ➢ Explants include cotyledon, leaf, thin tissue layer, peduncle, hypocotyls, stem, microspores

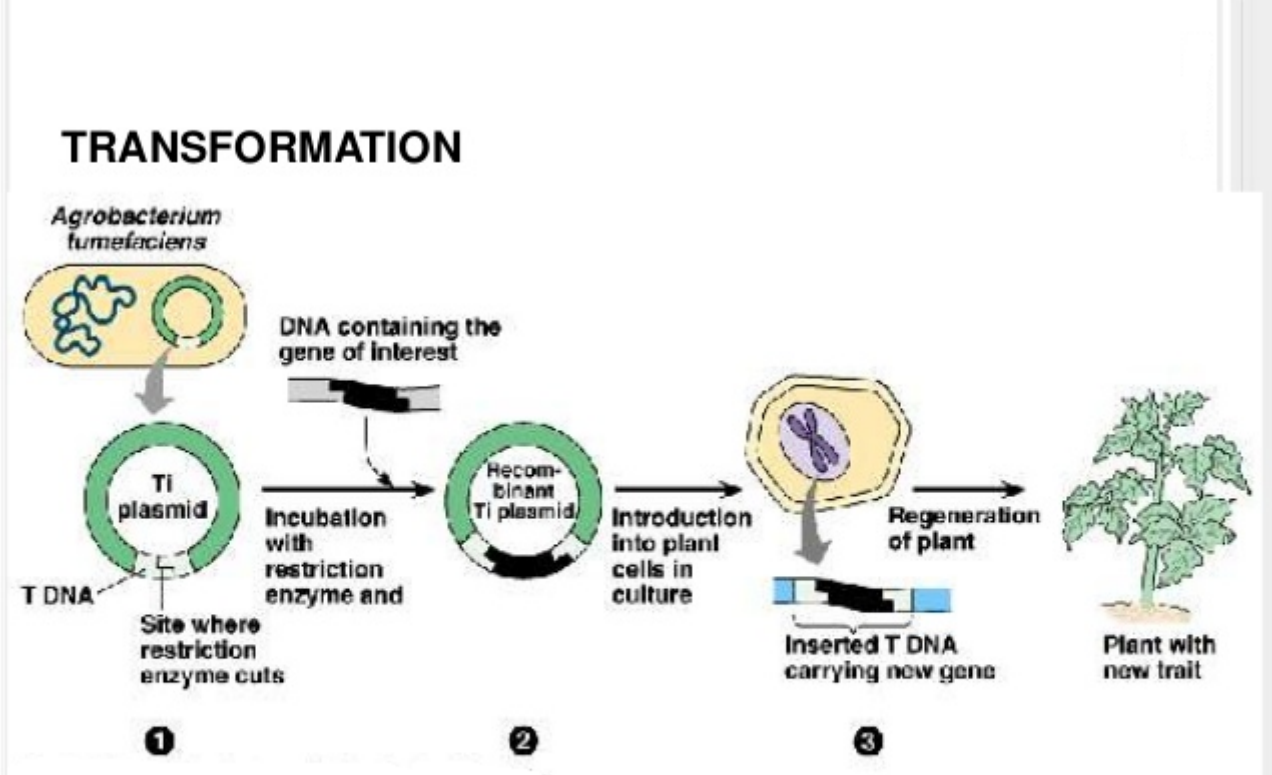


Insect, Virus and Herbicide Resistant Plant:

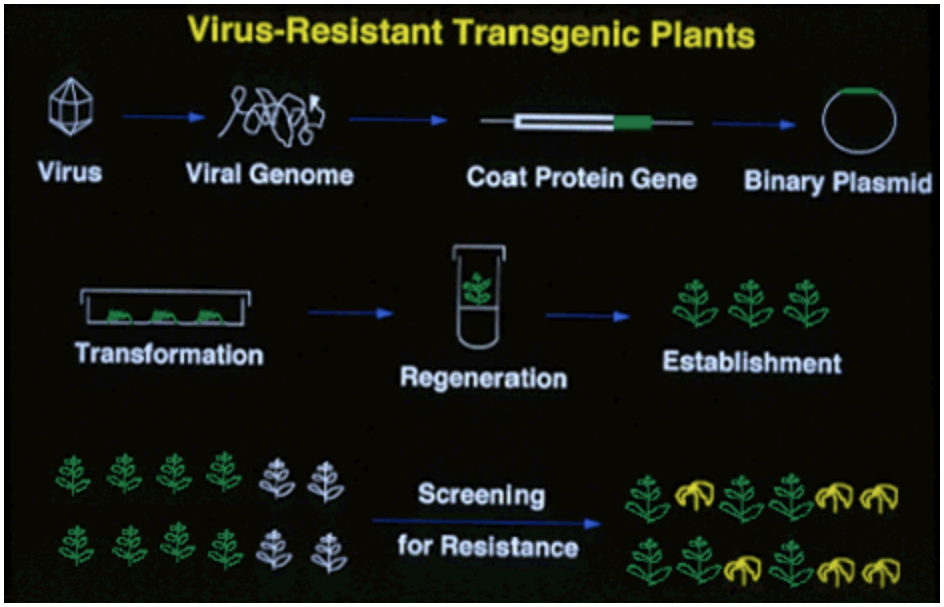


Insect resistant transgenic plants:

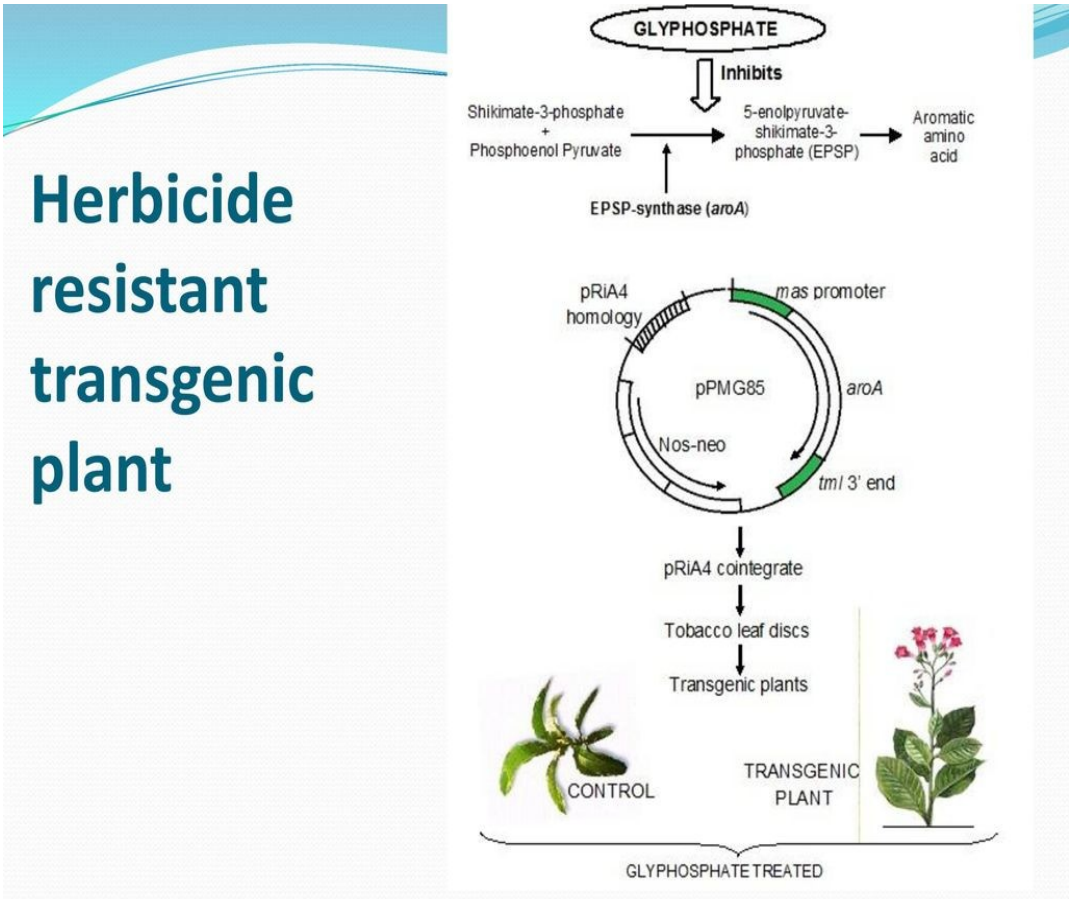
Crops that either naturally or through genetic engineering are able to resist **insect** damage. **Insect-resistant crops** generally produce compounds that are toxic to **insects** that attempt to eat the **resistant plants**.



Virus Resistant Plants:



Herbicide Resistant Plant:



Transgenic animals:

Transgenic animals are **animals** (most commonly mice) that have had a foreign gene deliberately inserted into their genome. Such **animals** are most commonly created by the microinjection of DNA into the pronuclei of a fertilised egg which is subsequently implanted into the oviduct of a pseudopregnant surrogate mother.

Methods of creation of transgenic animals

The three principal methods used for the creation of transgenic animals are **DNA microinjection, embryonic stem cell-mediated gene transfer** and **retrovirus-mediated gene transfer**.

Transgenic Animals:

A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome. The foreign gene is constructed using recombinant DNA methodology. In addition to the gene itself, the DNA usually includes other sequences to enable it

- to be incorporated into the DNA of the host and
- to be expressed correctly by the cells of the host.

- Transgenic sheep and goats have been produced that express foreign proteins in their milk.

- Transgenic chickens are now able to synthesize human proteins in the "white" of their eggs.

An example:

Normal mice cannot be infected with polio virus. They lack the cell-surface molecule that, in humans, serves as the receptor for the virus. So normal mice cannot serve as an inexpensive, easily-manipulated model for studying the disease. However, transgenic mice expressing the human gene for the polio virus receptor

- can be infected by polio virus and even
- develop paralysis and other pathological changes characteristic of the disease in humans.

Two methods of producing transgenic mice are widely used:

- Transforming **embryonic stem cells** (**ES** cells) growing in tissue culture with the desired DNA;
- Injecting the desired gene into the **pronucleus** of a fertilized mouse egg.

Method 1: The Embryonic Stem Cell Method

Embryonic stem cells (**ES** cells) are harvested from the **inner cell mass** (ICM) of mouse blastocysts. They can be grown in culture and retain their full potential to produce all the cells of the mature animal, **including its gametes**.

1. Make your DNA

Using recombinant DNA methods, build molecules of DNA containing

- the gene you desire (e.g., the insulin gene);
- **vector** DNA to enable the molecules to be inserted into host DNA molecules;
- **promoter and enhancer sequences** to enable the gene to be expressed by host cells.

2. Transform ES cells in culture

Expose the cultured cells to the DNA so that some will incorporate it.

3. Select for successfully transformed cells.

4. Inject these cells into the inner cell mass (ICM) of mouse blastocysts.

5. Embryo transfer

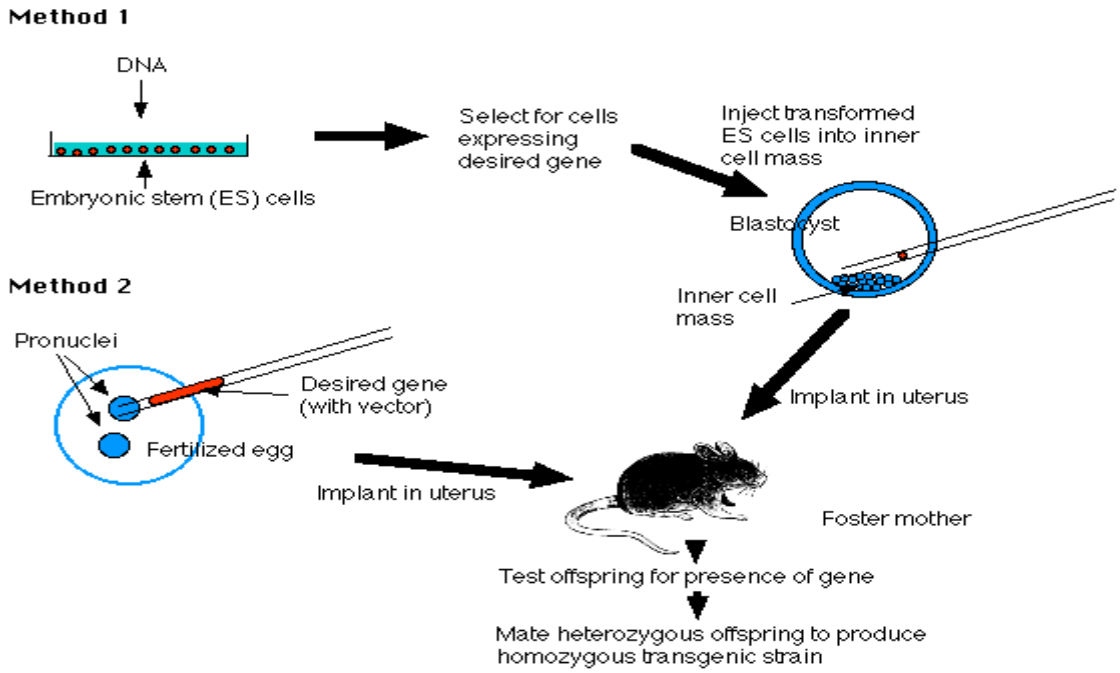
- Prepare a **pseudopregnant** mouse (by mating a female mouse with a vasectomized male). The stimulus of mating elicits the hormonal changes needed to make her uterus receptive.
- Transfer the embryos into her uterus.
- Hope that they **implant** successfully and develop into healthy pups (no more than one-third will).

6. Test her offspring

- Remove a small piece of tissue from the tail and examine its DNA for the desired gene. No more than 10–20% will have it, and they will be heterozygous for the gene.

7. Establish a transgenic strain

- Mate two heterozygous mice and screen their offspring for the 1 in 4 that will be **homozygous** for the transgene.
- Mating these will found the transgenic strain.



Method 2:The Pronucleus Method

1. Prepare your DNA as in Method 1

2. Transform fertilized eggs

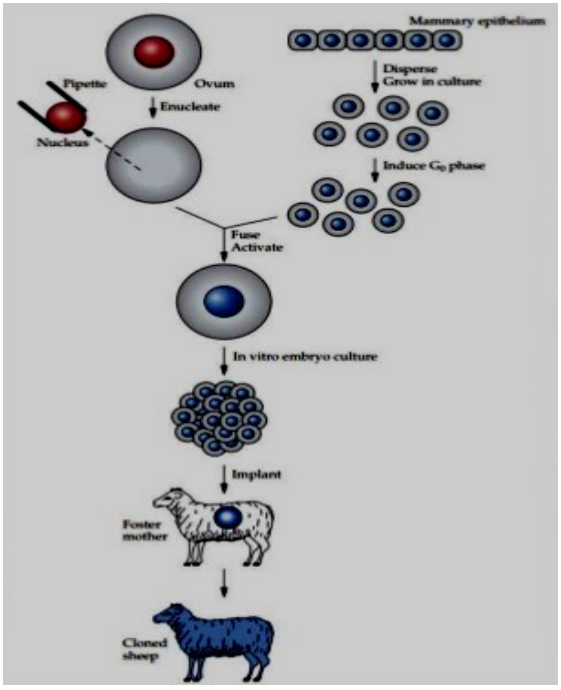
- Harvest freshly fertilized eggs before the sperm head has become a pronucleus.
- Inject the male pronucleus with your DNA.
- When the pronuclei have fused to form the diploid zygote nucleus, allow the zygote to divide by mitosis to form a 2-cell embryo.

3. Implant the embryos in a pseudopregnant foster mother and proceed as in Method 1.

Transgenic Sheep:

Dolly Sheep

Dolly the sheep was the first mammal to be cloned from an adult cell. In this, the udder cells from a 6-year-old Finn Dorset white sheep were injected into an unfertilized egg from a Scottish Blackface ewe, which had its nucleus removed. The cell was made to fuse by electrical pulses. After the fusion of the nucleus of the cell with the egg, the resultant embryo was cultured for six to seven days. It was then implanted into another Scottish Blackface ewe which gave birth to the transgenic sheep, Dolly.



Applications of Transgenic Animals

1. Normal Physiology and Development

In transgenic animals, a foreign gene is introduced due to which the growth factor is altered. Hence, these animals facilitate the study of gene regulation and their effect on the everyday functions of the body.

2. Study of Diseases

Transgenic animals are specially designed to study the role of genes in the development of certain diseases. Moreover, in order to devise a cure for these diseases, the transgenic animals are used as model organisms. These transgenic models are used in research for the development of medicines. For example, we have transgenic models for diseases such as Alzheimer's and cancer.

3. Biological Products

A number of biological products such as medicines and nutritional supplements are obtained from transgenic animals. Research for the manufacture of medicines to treat diseases such as phenylketonuria (PKU) and hereditary emphysema is going on. The first transgenic cow, Rosie, in 1997, produced milk which was rich in human protein (2.4 grams per litre). This milk contains the human gene alpha-lactalbumin and could be given to babies as an alternative to natural cow milk.

4. Vaccine Safety

Transgenic animals are used as model organisms for testing the safety of vaccines before they are injected into humans. This was conventionally done on monkeys.

Human Gene Therapy:

Gene therapy is a technique in which a functioning **gene** is inserted into a **human** cell to correct a **genetic** error or to introduce a new function to the cell. Many methods, including retroviral vectors and non-viral vectors, have been developed for both ex vivo and in vivo **gene** transfer into cells.

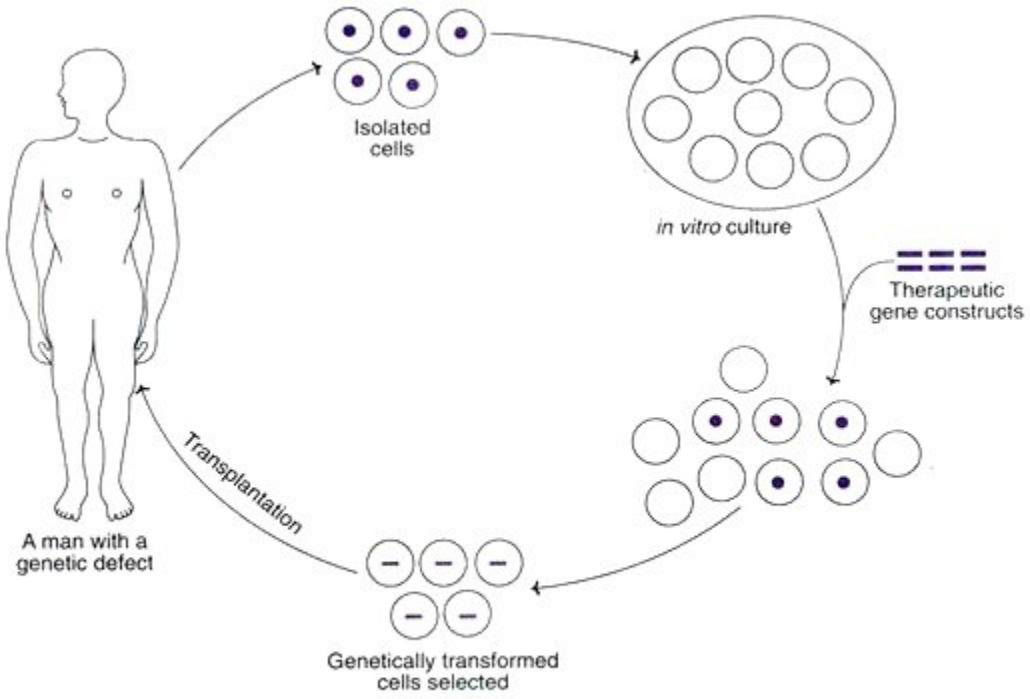
Types of gene therapy.:

The two types are:

- (1) **Ex Vivo Gene Therapy –** This involves the transfer of genes in cultured cells (e.g., bone marrow cells) which are then reintroduced into the patient.
- (2) **In Vivo Gene Therapy** - The direct delivery of genes into the cells of a particular tissue is referred to as in vivo gene therapy.

1. **Ex Vivo Gene Therapy:**

The ex vivo gene therapy can be applied to only selected tissues (e.g., bone marrow) whose cells can be cultured in the laboratory. The technique of ex vivo gene therapy involves the following steps



1. Isolate cells with genetic defect from a patient.

2. Grow the cells in culture.

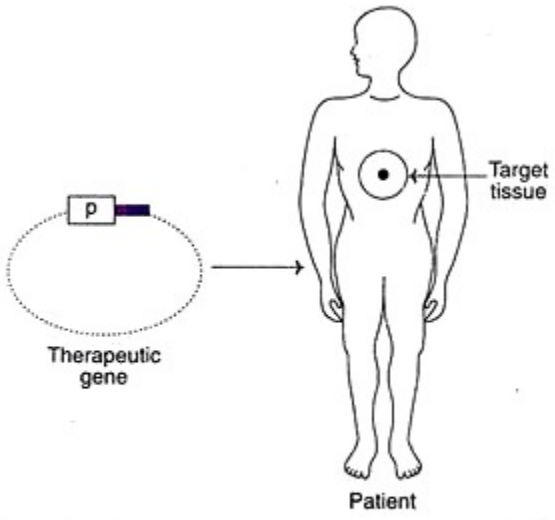
3. Introduce the therapeutic gene to correct gene defect.

4. Select the genetically corrected cells (stable trans-formants) and grow.

5. Transplant the modified cells to the patient.

2. In Vivo Gene Therapy:

The direct delivery of the therapeutic gene (DNA) into the target cells of a particular tissue of a patient constitutes in vivo gene therapy . Many tissues are the potential candidates for this approach. These include liver, muscle, skin, spleen, lung, brain and blood cells. Gene delivery can be carried out by viral or non- viral vector systems. The success of in vivo gene therapy mostly depends on the following parameters



Diagrammatic representation of in vivo gene therapy. (p-Promoter gene specific for therapeutic gene)

Applications:

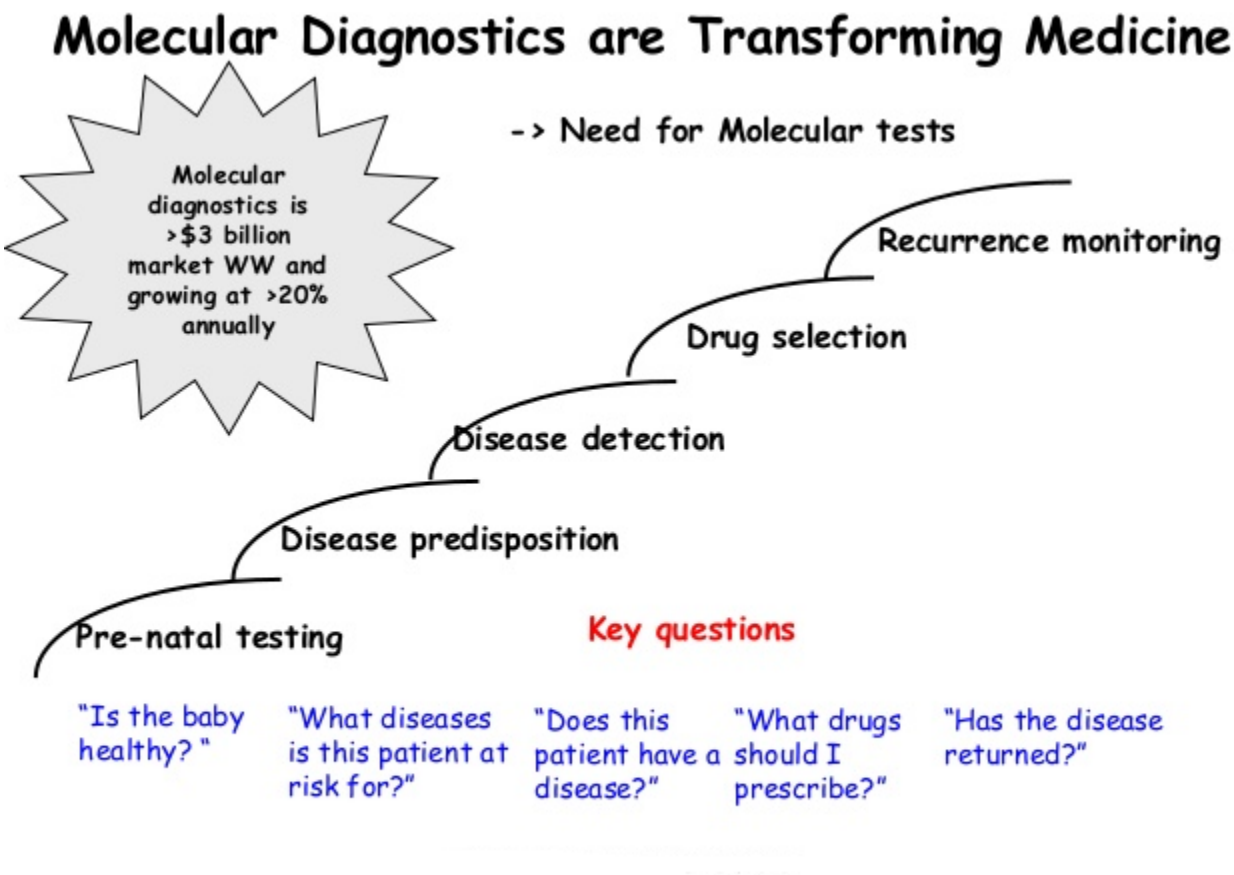
Gene therapy can deliver to target cells **genes** that code for the missing biological factor. Cancer, infectious diseases, cardiac disease, neurological disorders and some inherited conditions are among the areas into which **gene therapy** research is being carried out.

Molecular Diagnosis:

The use of molecular biology techniques to expand scientific knowledge of the natural history of diseases, identify people who are at risk for acquiring specific diseases, and diagnose human diseases at the molecular level.

Molecular Diagnostics Significance:

To face the near future, the medical practitioner not only understand molecular biology, but must also embrace the use of this rapidly expanding body of information in his medical practice, whether practicing family medicine pediatrics, oncology, obstetrics and gynecology, pathology, or any other medical specialty.



Characteristics of a Detection System:

• A good detection system should have 3 qualities:

- Sensitivity
- Specificity
- Simplicity

• **Sensitivity** means that the test must be able to detect very small amounts of target even in the presence of other molecules.

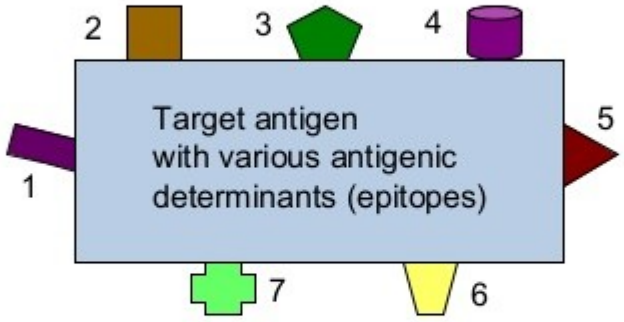
• **Specificity**: the test yields a positive result for the target molecule only.

• **Simplicity:** the test must be able to run efficiently and inexpensively on a routine basis.

Molecular Diagnostics Immunological Diagnostics Methods

1. Radioimmunoassay
2. Enzyme-Linked ImmunoSorbent Assay (ELISA)
3. Western Blotting
4. Immunoprecipitation
5. Immunofluorescence
6. Flow Cytometry and Fluorescence
7. Alternatives to Antigen-Antibody Reactions
8. Immunoelectron Microscopy

Target antigens and polyclonal versus monoclonal antibodies



Polyclonal antibodies are made against and react with multiple antigenic sites (epitopes) on a target antigen. Monoclonal antibodies are directed against a particular antigenic site.

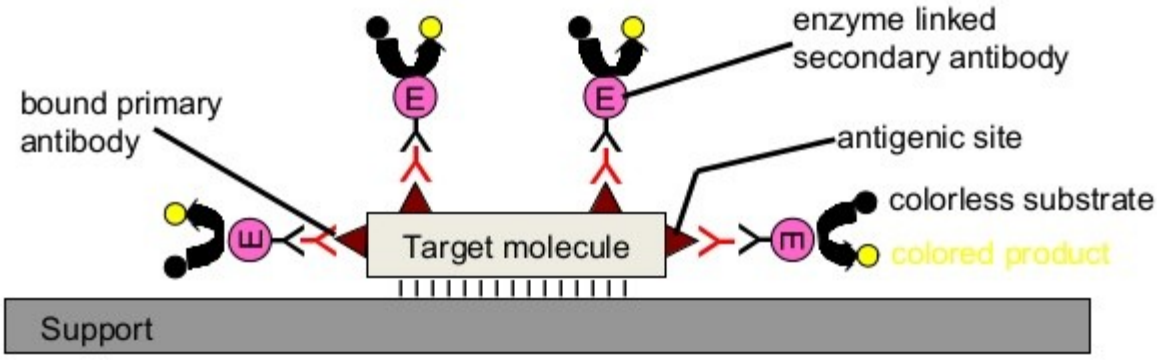
Enzyme-Linked Immunosorbent Assay (ELISA): Immunological detection

A. Bind sample to the support (commonly plastic or a membrane)

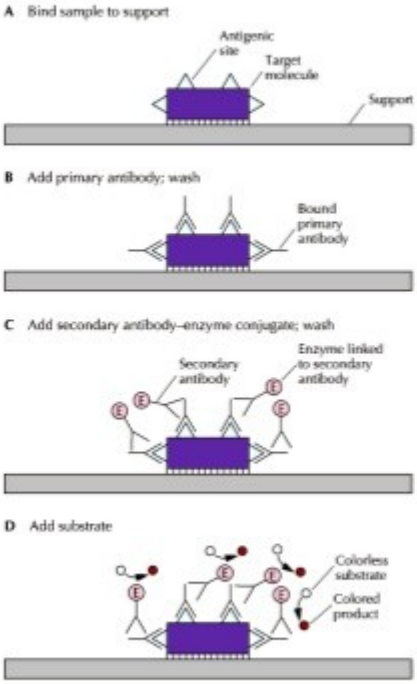
B. Add primary antibody; wash

C. Add secondary antibody-enzyme conjugate; wash

D. Add substrate

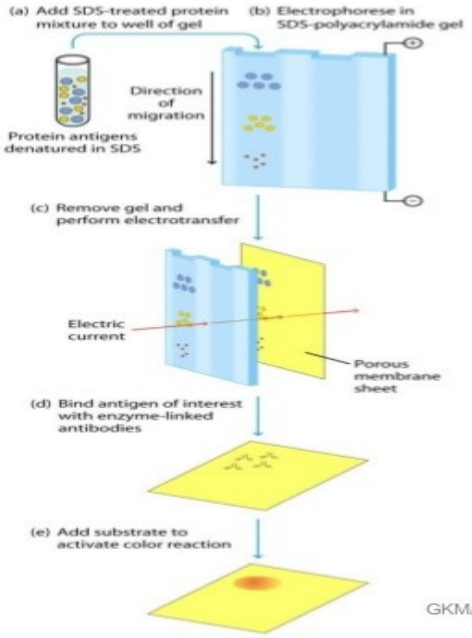


Immunological Diagnostics Methods - ELISA



- Addition of a **specific antibody** (primary antibody) which will bind to the test molecule if it is present.
- **Washing** to remove unbound molecules.
- Addition of **secondary antibody** which will bind to the primary antibody.
- The secondary antibody usually has attached to it an **enzyme** e.g. **alkaline phosphatase**.
- **Wash** to remove unbound antibody.
- Addition of a **colourless substrate** which will react with the secondary antibody to give a **colour reaction** which indicates a positive result.

-> can be used for quasi High-throughput!!!



Western blot

SDS-Page: separates the components according to their molecular weight.

Blot: the proteins in the gel are transferred to the sheet of nitrocellulose or nylon by the passage of an electric current.

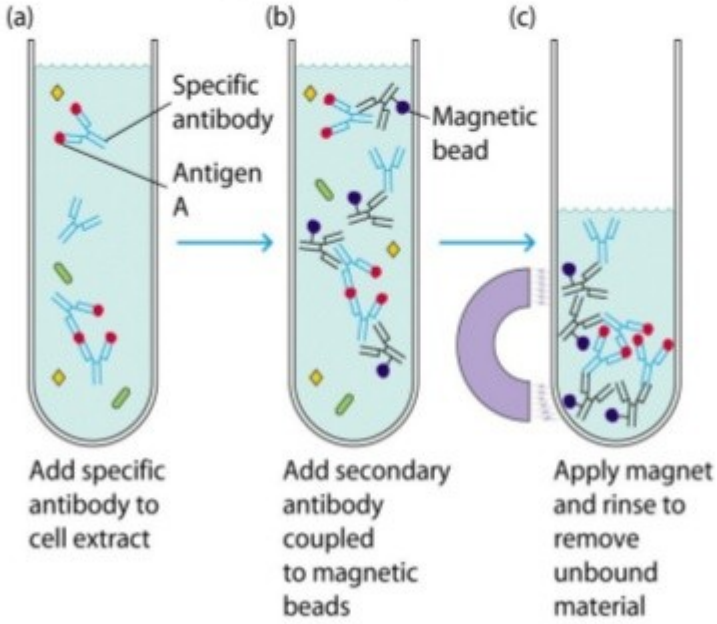
Immunoreaction: probed with Ab & then radiolabeled or enzyme-linked 2nd Ab.

Detection: a position is visualized by means of an ELISA reaction.

GKM/M.MEDPAEDS/LECT 01/2015

Immunoprecipitation

Immuno-precipitates can be collected using magnetic beads coupled to a secondary antibody.



UNIT V:

INTELLECTUAL PROPERTY (IPR)

In common sense intellectual property is a product of mind. It is similar to the property (**consisting of movable and immovable thing**) like a house or car where in the property or owner may use his property as his wishes nobody else can use his property without his permission as per Indian law.

Types of Intellectual Property

- Patents
- Copyright
- Trademarks
- Related Rights
- Geographical Indications
- Industrial Designs
- Unfair Competition
- Enforcement of Intellectual Property Rights
- Emerging Issues in Intellectual Property
    1. Biotechnology
    2. Traditional Knowledge

Patents

A patent is a government granted and secured legal right to prevent other forms of making, using or selling the invention covered by the patent. A patent is a personal property which can be licensed or sold by the person/organization like any other property.

Examples:

- Electric lighting- patents held by Edison and Swan
- Plastic- patents held by Baekeland
- Ballpoint pens- patents held by Biro
- Microprocessors- patents held by Intel.
- Telephones-patents held by Bell

Patents for:

- **The drug substance itself:**

        - Chemical composition of the API
    - **Method of use:**
        - Use of the drug to treat a particular condition
    - **The formulation:**
        - The physical form of a drug and method of administration
    - **The process of making it:**
        - Manufacturing methods

Copyright

Copyright aims at providing protection to authors (writers, artists, music composers, etc) on their creations. Such creations are usually designated as 'works".

The best example of copyright is the authored and edited books, or audio and video cassettes, which cannot be reproduced without the permission of the person (author, editor or publisher), who holds the copyright. In biotechnology, the copyright may cover DNA sequence data which may be published.

Trademarks

A trademark is a sign that is used to identify certain goods and services as those produced or provided by a specific person or enterprises.

E.g. "DELL" is trademark that identifies goods (computers and computer related objects).

E.g. "CITY BANK" is a trademark that relates to services (banking and financial services).

Related Rights

Related rights provide protection to the following persons or organizations:

- **Performers** (actors, musicians, singers, dancers, or generally people who perform), in their performances
- **Producers of sound recordings** (for example, cassette recordings and compact discs) in their recordings and
- **Broadcasting organizations,** in their radio and television programs.

Sometimes, these rights are also referred to as neighboring rights.

Industrial Designs

An industrial design is the ornamental or aesthetic aspect of an article. The design may consist of three-dimensional features, such as the shape of an article, or two-dimensional features, such as patterns, lines or color.

Industrial designs are applied to a wide variety of products of industry and handicrafts such as technical and medical instruments, watches, jewelry, house ware, electrical appliances, vehicles, architectural structures, textile designs and other luxury items.

To be protected under most national laws, an industrial design must appeal to the eye. This means that an industrial design is primarily of an aesthetic nature, and does not protect any technical features of the article to which it is applied.

Unfair Competition

Unfair competition is generally understood as any act of competition that is contrary to honest practices in industrial or commercial matters.

A dishonest practice is not something that can be defined with precision.

The standard of fairness or honesty may change from country to country, as well as evolve with time. It is, therefore, difficult to attempt to encompass all existing acts of unfair competition in one definition.

Enforcement (a law) of Intellectual Property Rights

A publisher may own copyright in a book, which has been reproduced and sold without his or her consent, at a cut price.

A sound producer, who has invested large amounts of money, in terms of talent and technical skill, in producing records, sees that copies of it are sold on the market, at cheap prices, without his authorization.

Someone else's trade mark may have been used by a company on similar or identical goods of lesser quality, harming thus the reputation of the legitimate owner, and inflicting on him or her serious financial loss, let alone exposing customer's health to danger.

Somebody may be using the geographical denomination of "Roquefort" on cheese manufactured elsewhere than in the region of Roquefort in France, thus deceiving the consumers as well as taking away business from legitimate producers.

In all such cases intellectual property rights (i.e. copyright, related rights, trademarks and geographical indications) have been infringed. It is important that in such cases enforcement mechanisms be called into play to protect not only the legitimate interests of the rights of the owners, but also of the public.

Emerging Issues in Intellectual Property

Intellectual property plays an important role in an increasingly broad range of areas, ranging from the internet to health care, to nearly all aspects of science and technology, literature and the arts.

The following two topics, ***Biotechnology*** and ***Traditional Knowledge***, are now being discussed at length at the international arena.

❖ Biotechnology

Biotechnology is a field of technology of growing importance in which inventions may have a significant effect on our future, particularly in medicine, food, agriculture, energy and protection of the environment.

The science of biotechnology concerns living organisms, such as plants, animals, seeds and microorganisms, as well as biological material, such as enzymes, proteins and plasmids (which are used in "genetic engineering")

❖ Traditional Knowledge

Traditional knowledge-used here broadly to refer to tradition-based innovations and creations resulting from intellectual activity in the industrial, scientific, literary or artistic fields-had been largely over-looked in the IP community until quite recently.

It is now increasingly recognized that the economic value of traditional knowledge assets could be further enhanced by the use of IP.

IPR in India:

Patent Administration in India

The Head Office is in **Kolkata**

Four branches:

1. **Kolkata**
2. **Mumbai**
3. **Delhi**
4. **Chennai**

ETHICS:

The field of ethics (or moral philosophy) involves systematizing, defending, and recommending concepts of right and wrong behavior

BIOETHICS:

Bioethics is the study of the **ethical issues** emerging from advances in **biology** and **medicine**. It is also moral discernment as it relates to medical policy and practice. Bioethicists are concerned with the ethical questions that arise in the relationships among **life sciences, biotechnology, medicine, politics, law**, and **philosophy. It** includes the study of values **("the ethics of the ordinary")** relating to primary care and other branches of medicine.

- ❖ The term **"bioethics"** was introduced in the **70's** by **Van Rensselaer Potter** for a study aiming at ensuring the **preservation of the biosphere**
- ❖ It was later used to refer a study of the ethical issues arising from health care, biological and medical sciences

Some historical examples:

- ✪ Abortion
- ✪ Contraception
- ✪ Kidney dialysis machine (Who had the priority?)
- ✪ Organ transplant, artificial ventilator, and brain death
- ✪ *In vitro* fertilization (IVF)
- ✪ Cloning and stem cell research
- ✪ Genetic engineering

LEGALITY: Definition:

Legality can be defined as an act, agreement, or contract that is consistent with the law or state of being lawful or unlawful in a given jurisdiction.

PRINCIPLE OF LEGALITY:

- ❖ Legality is the legal ideal that requires all **law** to be clear, ascertainable and non-retrospective.

- It requires **decision makers to resolve disputes by applying legal rules** that have been declared beforehand, and not to alter the legal situation retrospectively by discretionary departures from established law.

- In **criminal law** it can be seen in the general prohibition on the imposition of criminal sanctions for acts or omissions that were not criminal at the time of their commission or omission.

- The principle is also thought **to be violated** when the sanctions for a **particular crime are increased with retrospective effect.**

In administrative law it can be seen in the desire for state officials to be bound by and apply the law rather than acting upon whim. As such advocates of the principle are normally against discretionary powers.

MORALITY:

- Morality speaks of a system of behavior in regards to standards of right or wrong behavior.
- The word carries the concepts of:

  (1) Moral standards, with regard to behavior;

  (2) Moral responsibility, referring to our conscience; and

  (3) A moral identity, or one who is capable of right or wrong action.

- Common synonyms include ethics, principles, virtue, and goodness. Morality has become a complicated issue in the multi-cultural world we live in today. Let's explore what morality is, how it affects our behavior, our conscience, our society, and our ultimate destiny.

Morality principles concerning the distinction between right and wrong or good and bad behaviour.

ETHICS:

- **Ethics:** "the **rules of conduct** recognized in respect to a **particular class of human actions** or a particular group, culture"

Ethics and **morals** relate to "right" and "wrong" conduct. While they are sometimes used interchangeably, they are different: **ethics** refer to rules provided by an external source, e.g., codes of conduct in workplaces or principles in religions. **Morals** refer to an individual's own principles regarding right and wrong.

COMPARISON CHART:

Ethics versus Morals comparison chart

	Ethics	Morals
What are they?	The rules of conduct recognized in respect to a particular class of human actions or a particular group or culture.	Principles or habits with respect to right or wrong conduct. While morals also prescribe dos and don'ts, morality is ultimately a personal compass of right and wrong.
Where do they come from?	Social system - External	Individual - Internal
Why we do it?	Because society says it is the right thing to do.	Because we believe in something being right or wrong.
Flexibility	Ethics are dependent on others for definition. They tend to be consistent within a certain context, but can vary between contexts.	Usually consistent, although can change if an individual's beliefs change.
The "Gray"	A person strictly following Ethical Principles may not have any Morals at all. Likewise, one could violate Ethical Principles within a given system of rules in order to maintain Moral integrity.	A Moral Person although perhaps bound by a higher covenant, may choose to follow a code of ethics as it would apply to a system. "Make it fit"
Origin	Greek word "ethos" meaning "character"	Latin word "mos" meaning "custom"
Acceptability	Ethics are governed by professional and legal guidelines within a particular time and place	Morality transcends cultural norms

BASIC PRINCIPLES IN BIOETHICS:

In bioethics they are **four basic principles** and they were proposed by **Beaucham and Childress (1979):**

- Autonomy
- Beneficence
- No malfeasance
- Justice

Bioethics we find several grounded ethical theories. Two of these are **deontological ethics** and **utilitarian ethics.**

1. **Deontological ethics**

   - Proposed by Immanuel Kant

   - It consists in that reason identifies actions like good or bad, independent of their consequences. (Importance / Effect of what has gone before)

2. **Utilitarian ethics**

   - Proposed by Jeremy Bentham and John Stuart-Mill

   - It says that actions are good or bad depend on their consequences. (Importance / Effect of what has gone before)

   - The balance between purposes that give benefits or damage is produced by utilitarian ethics.

These **principles can be grouped in two levels**:

- **Minimum levels:** obligations that generate universal duties and these involve negative transitive duties (facts that you cannot do other people). Here, there are principles of no malfeasance and justice.
- **Maximum levels:** they are related with the choice of the vital project that every person chooses to depend on their scale of values. They generate imperfect obligations: facts that I can auto impose, but I cannot call for other people (neither other people to me). Here, there are principles of autonomy and beneficence.

- **The Principle of Respect for autonomy:**

  Autonomy is Latin for "self-rule" We have an obligation to respect the autonomy of other persons, which is to respect the decisions made by other people concerning their own lives. This is also called the principle of human dignity. It gives us a negative duty not to interfere with the decisions of competent adults, and a positive duty to empower others for whom we're responsible.

  Corollary principles: honesty in our dealings with others & obligation to keep promises.

- **The Principle of Beneficence:**

  We have an obligation to bring about good in all our actions.

  Corollary principle? We must take positive steps to prevent harm. However, adopting this corollary principle frequently places us in direct conflict with respecting the autonomy of other persons.

- **The Principle of non malfeasance:**

  (It is not "non-malfeasance," which is a technical legal term & it is not "no malevolence," which means that one did not intend to harm.)

  We have an obligation not to harm others: "First, do no harm."

  Corollary principle: Where harm cannot be avoided, we are obligated to minimize the harm we do.

  Corollary principle: Don't increase the risk of harm to others.

  Corollary principle: It is wrong to waste resources that could be used for good.

  Combining beneficence and non malfeasance: Each action must produce more good than harm.

- **The Principle of justice**

  We have an obligation to provide others with whatever they are owed or deserve. In public life, we have an obligation to treat all people equally, fairly, and impartially.

  Corollary principle: Impose no unfair burdens.

  Combining beneficence and justice: We are obligated to work for the benefit of those who are unfairly treated.

OTHER IMPORTANT PRINCIPLES

There are other important principles in bioethics.

- **Fidelity (Faith / Loyal / Strict / Accuracy):** protection of people, based on caution, proportionality, no discrimination and respect for people's dignity. It includes privacy's protection and confidentiality, keeping the promises and commitment.
- **Transparency:** gives law and access to information. All information has to communicate clearly, comprehensively, honest and real.

- **Caution:** based on analysis of risks. All investigations that could put at risk people's health and future generations has to avoid.
- **Principle of proportionality:** it is related to the principle of beneficence and looks at the relationship between the benefit obtained and the "costs" of means, human and monetary resources, risks and what the negative effects are.
- **Principle of non-discrimination:** all persons who must be treated equally.
- **Principle of respect for dignity:** no one has to be subjected to humiliation, must receive help in situations of need, have a minimum quality of life without suffering and freedom of action and decision, and not be used as the purpose of others.
- **Principle of respect for privacy and confidentiality:** not unnecessarily reveal and/or interested personal and sensitive data concerning the subject. It is not an absolute principle and in front of a crime is not fulfilled.
- **Principle of respect for the right to information:** all those involved in the process must know all the information (before, during and after the investigation).
- **Principle of free participation and donation:** participation and donation are free and altruistic since if we are not talking about sale or exchange.

Risks and ethics of biotechnology:

The modern biotechnology deals with genetic manipulations of viruses, bacteria, plants, animals, fish and birds. Introduction of foreign genes into various organisms raises concerns about the safety, ethics and unforeseen consequences.

Some of the popular phrases used in the media while referring to experiments on recombinant DNA technology are listed:

i. Manipulation of life

ii. Playing with God

iii. Man-made evolution

The major apprehension of genetic engineering is that through recombinant DNA experiments unique microorganisms or viruses (either inadvertently or sometimes deliberately for the purpose of war) may be developed that would cause epidemics and environmental catastrophes. Due to these fears, the regulatory guidelines for research dealing with DNA manipulation were very stringent in the earlier years.

So far, risk assessment studies have failed to demonstrate any hazardous properties acquired by host cells/organisms due to transfer of DNA. Thus, the fears of genetic manipulations may be unfounded to a large extent. Consequently, there has been some relaxation in the regulatory guidelines for recombinant DNA research. It is now widely accepted that biotechnology is certainly beneficial to humans. But it should not cause problems of safety to people and environment, and create unacceptable social, moral and ethical issues.

Biotechnology largely contributes to human genetic research involving the following areas:

i. Genetic testing and screening for diseases

ii. Genetic portfolios

iii. Human gene therapy.

Genetic testing and screening:

Techniques are now available for prenatal testing to specifically detect whether a fetus carries genetic defects. This will help the parents to be better prepared for the future baby. The negative aspect of prenatal testing is that the couple may opt for abortion even for a minor genetic defect or sometimes for gender bias.

Genetic portfolios:

The elucidation of the entire human genome sequence and identification of genes has now become a reality. It may soon be possible to have individual genetic portfolios that will diagnose future health complications e.g. risk for cancer, heart disease. The genetic portfolios (based on the genes) will foretell the individuals' future which is now being predicted through stars (astrology).

Genetic portfolios of individual may pose certain problems with regard to marriages, insurances. Who would like to be a spouse of someone who will soon be a victim of cancer or heart attack? Which insurance company would insure a person with a very high risk of diseases? Many ethical committees are of the opinion that insurance companies should not require, or should not be allowed to have access to individuals' genetic portfolios.

Human gene therapy:

Theoretically, correction of genetic defects is possible by gene therapy. The present status of human gene therapy has been described. From the ethical perspective, gene therapy involving introduction of genes into a patient is comparable to the practice of transplantation of organs (e.g., heart, liver, lungs).

Therefore, there is not much controversy over gene therapy, as long as it is intended to be used to alleviate serious medical disorders. However, the gene therapy must be under a close supervision to satisfy medical, legal, ethical and safety implications, besides addressing the public concerns.

Animal ethics

- **Animal ethics** is a term used in academia to name the branch of ethics that examines human-animal relationships, the moral consideration of animals and how nonhuman animals ought to be treated.
- The subject matter includes animal rights, animal welfare, animal law, speciesism, animal cognition, wildlife conservation, the moral status of nonhuman animals, the concept of nonhuman personhood, human exceptionalism, the history of animal use, and theories of justice.

- Several different theoretical approaches have been proposed to examine this field, in accordance to the different theories currently defended in moral and political philosophy

Ethical Guidelines for Animal Research

There are a wide range of ethical assessments regarding animals used in research. There are general opinions on that animals do have a moral status and how they are treated should be subjected to ethical consideration. Some of the positions include:

- Animals have intrinsic values that must be respected.
- Animals can feel pain and their interests must be taken into consideration.
- Our treatment of all animals/lab animals reflects on our attitudes and influences us on our moral beings.

To go into further details of guidelines that are to be followed includes:

1. **Respect Animal Dignity:** Researchers must have respect towards the animals' worth, regardless of their value and the animals' interests as living, sentient creatures. Researchers has to have respect when choosing their topics/methods, and when expanding their research. Researchers also has to supply care that is adapted to needs to each laboratory animal.
2. **Responsibility for considering options (*Replace*):** When there are alternatives available, researchers are responsible for studying those alternatives for animal experimentation. When there are no good alternatives available, researchers have to consider if the research can be postponed until a good alternative are developed. While being able to justify the experiments on animals, researchers then have to be accountable for the absence of alternative options and the urge to obtain the knowledge immediately.

3. **The principle of proportionality: responsibility for considering and balancing suffering and benefit:** Researchers have to consider both the risks of pain and suffering that laboratory animals will face and assess them in the value of relationship to the research of animals, people, and the environment. Researchers have a responsibility on whether or not the research will have improvements for the animals, people or the environment. All of the possible benefits of the study has to be considered, substantiated and specified in both the short and long run. This responsibility also entails the obligation to consider both the scientific quality of the experiment and whether or not the experiment will have relevant scientific benefits. Suffering can only be caused by animals if there is a counterbalance of a substantial and probable benefits for animals, people or the environment. Since there are many methods of analyzing the harm and the benefits, research institutions have to provide training on suitable models and researchers have the responsibility to use the methods of analysis when planning any experiments on animals.

4. **Responsibility for considering reducing the number of animals (*Reduce*):** Researchers have the responsibility to consider whether or not its acceptable to reduce the amount of animals that an experiments plan on using and include the number necessary to both the scientific quality of the experiments and the relevance to the results only. Before the experiment, researchers have to conduct reading studies and consider alternative designs and perform the calculations that are needed before beginning an experiment.

5. **Responsibility for minimizing the risk of suffering and improving animal welfare (*Refine):*** Researchers have the responsibility to assess the expected effect on laboratory animals. Researchers have to lessen the risk of suffering and provide excellent animal welfare. Suffering includes pain, hunger, malnutrition, thirst, abnormal cold/heat. fear, stress, illness, injury, and restrictions to where the animal can't be able to behave naturally and normally. To find out what is a considerable amount

of suffering, a researcher's assessment should be based on which animal suffers the most. Considering the animals is the deciding factor if there are any doubts about regarding the suffering the animals will face. Researchers have to consider the direct suffering that the animal might endure during an experiment, but there are risks before and after the suffering, including breeding, transportation, trapping, euthanizing, labeling, anesthetizing, and stabling. This means that all the researchers has to take into account of the needs of periods for adaptation before and after an experiment.

6. **Responsibility for maintaining biological diversity:** Researchers are also responsible for ensuring that the use of laboratory animals don't disrupt or endanger biological diversity. This means that researchers have to consider the consequences to the stock and their ecosystem as a whole. The use of endangered species have to be reduced to a minimum. When there is credible and uncertain, knowledge that the inclusion of animals in research and the use of certain methods may have ethically unacceptable consequences for the stock and the ecosystem as a whole, researchers must observe the precautionary principle.

7. **Responsibility when intervening in a habitat:** Researchers have a responsibility for reducing the disruption and any impact of the natural behaviors of the animals, including those who aren't a direct test subjects in research, as well as the population and their surroundings. Most research and technology-related projects, like the ones regarding environmental technology and surveillance, might impact the animals and their living arrangements. In those cases, researchers have to seek to observe the principle of proportionality and to decrease possible negative impact

8. **Responsibility for openness and sharing of data and material:** Researchers have the responsibility for ensuring the transparency of the research findings and facilitating sharing the data and materials from all animal experiments. Transparency and sharing are important in order to not repeat the same experiments on animals. Transparency is also important in order to release the data to the public and a part of researchers' responsibility for dissimulation. Negative results of the experiments on animals have should be public knowledge. Releasing negative results to other researchers could give them more on the information about which experiments that are not worth pursuing, shine a light on unfortunate research designs, and can help reduce the amount of animals use in research.

9. **Requirement of expertise on animals:** Researchers and other parties who work and handle live animals are required to have adequately and updated documentation expertise on all animals. This includes knowledge about the biology of the animal species in question, and willingly be able to take care of the animals properly.

10. **Requirement of due care:** There are many laws, rules, international convention, and agreements regarding the laboratory animals that both the researchers and the research managers have to comply with. Anyone who wants to use animals in experiments should familiarize themselves with the current rules.

Major beginning-of-life issues

The moral status of human life before birth. Although none dispute that, in a biological sense, the embryo is alive and has a distinct genetic identity from the moment of conception, major religious traditions differ on the moral status they accord to the embryo and fetus. This profoundly influences the attitudes that they take toward many technologies and techniques. Some traditions, especially Roman Catholicism and some other Christian denominations, believe that the embryo has the status of a full human person from the moment of conception. Others, including Islam and Judaism, recognize that the embryo and fetus have the potential for full human life, which they hold in reverence, but do not accord parity with people who have been born. Some Christians share this view. Others believe that human status begins at some intermediate point, as, for example, when the fertilized ovum implants in the uterus or when the fetus is considered capable of viability outside the womb. Within a number of larger religious traditions, furthermore, differences exist in interpretation of the group's larger orientation toward the moral status before birth.

Embryonic stem cell research. The moral status accorded to the embryo and fetus profoundly affects how various traditions view specific techniques and technologies, such as research on stem cells derived from embryos, which some religious groups encourage as a potentially valuable health measure and others reject as homicide.

Assisted reproduction. Religious traditions differ in their attitude toward techniques to assist reproduction that involve creation or manipulation of embryos or use of donor eggs or sperm. Some approve these methods and others forbid them.

Cloning of human embryos. Faith communities in the United States universally reject cloning of embryos for the purpose of producing human beings who will be born. Some however, accept cloning for the purpose of producing products, such as stem cells, for use in medical treatment, a process known as therapeutic cloning, while others categorically reject the practice.

Conflict between the welfare of the mother and of the pregnancy. Pregnancy can present medical issues in which the health or welfare of the mother is at odds with those of the pregnancy or of the embryo or

fetus, such as when the mother has or develops a condition that makes the pregnancy or birth a threat to her health or life. Sometimes saving a problematic pregnancy also can require treatments that may be detrimental to the mother. Religious traditions differ on how to weigh the conflicting interests in such cases. Some, particularly some Christian denominations, believe that the interests of the embryo or fetus are paramount most or all of the time. Others, such as Judaism, believe that the life of the mother should take precedence throughout the pregnancy and birth.

Prenatal diagnosis and resultant abortion. Genetic and other technologies permit diagnosis of various diseases and conditions that can threaten the life, health or normal development of the future baby. This leads to the question of whether to terminate the pregnancy to forestall the suffering of the child and the family if the child is born. Some traditions forbid termination and therefore may encourage expectant parents to forgo prenatal testing. Others permit or even encourage these practices in order to spare a baby and family from suffering a grave or inevitably fatal disease.

Decisions regarding the birth and care of extremely premature babies or those with severe birth defects. Babies born extremely premature or with severe birth defects have a high likelihood of dying, and, should they survive, of suffering severe ailments or disabilities. Heroic neonatal intensive care techniques are often capable of keeping such babies alive, but, in many cases, without the assurance or possibility of anything close to normal development. The type and degree of care that should be given to a child whose extreme immaturity or birth defects are likely to result in major suffering are extremely controversial, both among and within religious groups and within the medical community. In many cases, the principles and considerations appropriate to end-of-life care become relevant, but with the complication that the patient is unable to express a preference.

Euthanasia

Euthanasia (from Greek: "good death": *eu*; "well" or "good" +, *thanatos*; "death") is the practice of intentionally ending a life to relieve pain and suffering.[Different countries have different euthanasia laws. The British House of Lords Select Committee on Medical Ethics defines euthanasia as "a deliberate intervention undertaken with the express intention of ending a life, to relieve intractable suffering".In the Netherlands and Belgium, euthanasia is understood as "termination of life by a doctor at the request of a patient".The Dutch law, however, does not use the term 'euthanasia' but includes the concept under the broader definition of "assisted suicide and termination of life on request".Euthanasia is categorizedin different ways, which include voluntary, non-voluntary, or involuntary:[

- Voluntary euthanasia is legal in some countries.
- Non-voluntary euthanasia (patient's consent unavailable) is illegal in all countries.

- Involuntary euthanasia (without asking consent or against the patient's will) is also illegal in all countries and is usually considered murder.

Voluntary euthanasia

Voluntary euthanasia is conducted with the consent of the patient. Active voluntary euthanasia is legal in Belgium, Luxembourg and the Netherlands. Passive voluntary euthanasia is legal throughout the US per *Cruzan v. Director, Missouri Department of Health*. When the patient brings about their own death with the assistance of a physician, the term assisted suicide is often used instead. Assisted suicide is legal in Switzerland and the U.S. states of California, Oregon, Washington, Montana and Vermont.

Non-voluntary euthanasia

Non-voluntary euthanasia is conducted when the consent of the patient is unavailable. Examples include child euthanasia, which is illegal worldwide but decriminalised under certain specific circumstances in the Netherlands under the Groningen Protocol.

Involuntary euthanasia

Involuntary euthanasia is conducted against the will of the patient.