Everwin Matric. Hr. Sec. School

Std:XII Minimal learning Material

Bio-Botany

Chap-4 Principles and processes of Biotechnology

The term Biotechnology -Karl Ereky

Biotechnology- Science of applied biological process.

Development of Biotechnology

Conventional (traditional Biotechnology)
- → Kitchen technology
- → Dairy products
- → alcoholic beverages

Modern biotechnology → ability to change the genetic material → r-DNA → Ownership of the newly developed technology → Pharmaceutical breweries, Agro Industries.

Major Focus of Biotechnology:

Fermentation - Production of Acids, Alcohols, Antibiotics

Biomass- Production of Scp, biofuel.

Enzymes- Biosensors.

Biofuels- Production of hydrogen, alcohol, Methane.

Microbial Inoculants - Bio fertilizer,

Plant, animal cell culture - Secondary metabolites monoclonal antibodies.

Process Engineering - Effluent treatment water recycling.

6000 BC-3000 BC - Bread making, Fermentation of juices using yeast.

Pre 20th Century:

Antoine Lavoisier - chemical basis of alcoholic fermentation.

Edward Jenner - First viral vaccine.

Gerardus Johannes Mulder and Jons Jacob Berzelius } Discovered and named Protein.

Ernst Hoppe Seyler - Discovered Enzyme Invertase (artificial sweetner)

Louis Pasteur - Indentified role of micro organisms in fermentation.

20th Century:

Term Biotechnology - Karl Ereky.

Pencillin Discovery - Alexander flemming.

One enzyme one gene hypothesis(Neurospora) - George Beadle and Edward Tatum.

DNA as the genetic material - Avery Macleod mccarty.

Double helix st-DNA - Watson and Crick

Restriction Enzymes - Arber, Smith, Nathans

r-DNA tech, modified gene - Stanley cohen , Annie chang, Robert Helling, Herbert Boyer.

Monoclonal antibodies - Kohler and Milstein

Sequence DNA technique - Sanger Gilbert.

Human Insulin in E.coli (Humulin)

Artificial gene - H.G. Khorana.

Ti plasmids usage.

PCR- Kary mullis

[Polymerase Chain reaction]

Gene transfer by biolistic transformation

First chromosomes of Yeast is sequenced

First Genetically modified Food - Flavour Sour tomato

Dolly first transgenic animal - Ian wilmet. (Nuclear cloning)

First plant genome - Arabidopsis thaliana sequenced.

21st Century:

HGP creates a draft of human genome sequence

HGP completed in the Year 2003

46 chromosomes of human.

Invitro fertilization - Sir Rober. G Edwards in animal.

Stem cell therapy (2016) → Stroke → Walk patients

Blood stem cells grown (2017) (2018)

Discovered protein found - James Allison and Tasuku Honjoin in immune cells

Traditional Boitechnology:

→ Kitchen technology - Fermenting bacteria

Fermentation: Latin → Fervor → to boil.

→ Metabolic process in which organic molecules are converted into acids, gases or alcohol in the absence of O_2.

→ Study of fermentation - Zymology.

→ Louis Pasteur demonstrated fermentation was caused by yeast.
→ Beverage Industries, Leavening of bread, production of organic acids to Preserve flavor vegetables and dairy products.

Bioreactor (fermentor):

→ Container provide an optimum environment in which micro organisms or their enzymes interact with a substrate and produce the required product.

→ Agitation, temperature and p^H are controlled in the Bioreactor.

FERMENTATION PROCESS

Up Stream	Down Stream
Before starting in the Fermnter.	After the fermentation Process.
i.e Growth of Inoculum sterilization	Distillation, Centrifuging, Filtration,Solvent entraction, purification

Procedure of Fermentation:

→ Selection of bio reaction based on the product.
→ Suitable substrate in liquid media is added.
→ Temperature, p^H Controlled.
→ Organism is added.
→ Incubated at a specific temperature and time[aerobic anaerobic]
→ with drawal of product.

Application of fermentation in Industries:

1. Microbial biomass production:
   Bio mass - SCP [algae, bacteria, yeast funi are grown, dried]- Human Food.
2. Microbial Metabolites:
   Compounds from microbes, useful to man and animals.

Microbial Metabolites

Primary	Secondary
Produced for the maintenance of life	Nor required for the vital life process.
Eg: Ethanol, Citric acid	Eg: Amphotericin B — Streptomyes nodosus
	pencillin — Penicillium chrysogeneum
	Streptomycin — S. grises
	Tetra cycline — S. aureofacins
	Toxins, vitamins, alkaloids

3. Microbal enzymes:
   Microbes secrete enzymes into the growth media used in detergents, brewing, food processing, pharmaceutical.
   eg: Lipase, Isomerase.
4. Bio conversion, Bio transformation or modification of the substrate
   →Microbes has the capacity to produce valuable products conversion of ethanol to vinegar.
   Isoproponal to acetone sorbitol to sorboce, sterol to steroid.

SCP: Single cell protein

→ Dried cells of microbes.
→ Higher protein, vitamin, amino acid and liquid content.
→ High nucleic acid content, slower in digestibility.

Microbes in SCP production.

Bacteria: Methylophilus methylotrophus cellulomonas, Alcaligenes
Fungi: Agaricus campestris, Candida utilis Saccharomyces cerevisiae.
Algae: Spirulina, Chlorella, Chlamydomonas.

→ SCP is used by Astronauts and Antartica expedition scientists.
→ Spirulina can be grown from waste water from potato processing plants, straw, molasses.
→ 250g of Methylophilus methyotrophus as its high state of biomass production and growth can be expected to produce 25 tonnes of protein.

Application of Biotechnology:

→ Protein supplement
→ Cosmetic products,
→ Feeding poultry, cattle, fishes.
→ Emulsifying agents aroma carriers.
→ Paper, leather processing as foam stabilizers.

Advancements in Modern Biotechnology :

Gene manipulations, protoplasmic fusion

Genetic engineering: (rDNA technology or gene manipulation gene cloning)

→ Recombination carried out artificially using modern technology is called r-DNA technology.

→ Transfer of specific gene from one organism into another organism using vectors are instruments(electro poration, gene gun, liposome, chemical mediated, and micro injection).

Steps involved in Recombinant DNA:

Technology: Isolation of DNA fragment contain gene of Interest
↓
(Insert)
Generation of rDNA
Insertion of DNA to vector
↓
Selection of transformed host cells that is carrying rDNA, allows multiplication
↓
Large amount of rDNA expressed.

Whenever vectors are not involved PCR technique is used to multiply the desired gene.

PCR – Polymerase chain reaction use to make copies of a particular region of DNA

Tools for Genetic Engineering

Host Restriction Enzymes | DNA ligase | Alkaline phosphates | Vectors

Restriction Enzymes:

Escherichia Coli Enzymes

which added methyl group to DNA | Later-Restriction endonuclease

Responsible for restricting the growth of Bacteriophage

Restriction Sites:

Cleave DNA into fragments at or near specific recognition sites with in the molecules.

Restriction Enzymes

Exonuclease	Endonuclease
Remove nucleotides one at a time from the end of DNA molecule	Break internal phosphodiester bonds within a DNA
Eg: Bal 31, Exonuclease III	Eg: Hindi II, , EloRI PvalI, Tag I

Restriction Endonclease: Molecular scissor

Act as foundation of r-DNA technology'

Exist in many bacteria

Function as part of their defence mechanism, so called restriction modification system

Three main classes of restriction endonuclease

Based on mode of action: Type I | Type II | Type III

Preferred for use in r-DNA Tech cut (4-8bp)

Hind II → cut 6bp (Recognizing a specific sequence of base pairs → recognition sequence)

→ 900 Restriction enzymes have been Isolated from 230 strains

→ Naming of Restriction Endo nuclease by standard procedure

→ First letter – Genus name

Followed by the first two letter → species then comes – strain

Eg: ECORI (E) Escherichia Coli (Co)

RY13 (R) First end nuclease (I) to be discovered

→ ECORI contains Antibiotic resistance gene, recognition sites

Palindrome Repeats:

Symmetrical repeated sequence in DNA strands

5^1 CATTATATAATG3^1

3^1 GTAATATATTAC5^1

Cleavage by restriction enzyme

Blunt (flush end)	Sticky (cohesive end)
→ cleave both strands of DNA through centre	→ staggered or asymmetric cuts
→ symmetric cuts	→ protruding and recessed ends

DNA Ligase:

→ Joins the sugar and phosphate molecules of ds DNA with 5′-PO and 3′-OH in an ATP dependent reaction.

→ Isolated from T_4 phage

Alkaline Phosphates:

→ Adds or removes specific phosphate group at 51 of ds DNA or ss DNA or RNA

→ Prevents self ligation

→ purified from bacteria and calf intestine

→ DNA molecule → capable of self replication

Vectors:

[Plasmid), [Cloning DNA]

Vector

Cloning vector	Expression vector
→ used for cloning of DNA Insert	→ Used to express the DNA insert

Properties of Vectors:

→Able to replicate, produce multiple copies.

→should be small in size less than 10 kb.

→ Contain an origin of replication.

→ contain suitable marker, antibiotic resistance, target sites, restriction sites.

→ Ability to integrate with DNA insert .

→ Have more than one restriction site.

MCS (multiple cloning site) poly linker.

Features Required to facilitate cloning into a Vector:

1. Origin of replication (o.ri)
   → Sequence from where replication starts.
   → Piece of DNA when linked to this sequence can be made to replicate.
2. Selectable Marker:
   → Identifying, eliminating non transform ants and selectively permitting the growth of the transform ants.
3. Cloning sites:
   → To link the alien DNA vector needs few, Single recognition sites.

<u>Types of vector</u>:

Plasmid: Extra chromosomal self replicating as DNA, vector needs few, single recognition sites.

PBR 322 Plasmid:

→ Reconstructed Plasmid.

→ contains 4361 bp.

P- Plasmid

B- Boliver

R- Rodriguez

322 - number of plasmid developed from their laboratory.

→contains,ori,amp^R tet ^{R} [antibiotic resistance genes]

→ Recognition sites for restriction enzymes [Hind III, ECoR I, BamHI, SalI, PVU II, Pst I, Cla I]

Ti plasmid:

→ Found in Agro bacterium tumefaciencs.

→ Carries tra gene help to transfer T-DNA.

→ Has Onc gene - Oncogenecity.

→ Ori gene - Origin of replication

→ incgene - Incompatibility.

Agro bacterium→ used for introduction of genes of desirable traits into plants.

Transposon as Vector: (mobile elements)

→ DNA sequence able to insert without having any sequence relationship with the target locus [Jumping or Walking genes].

→ Used as genetic tool for analysis of gene and protein functions.

→ use of transponsons is well studied in Arabidopsis thaliana, Escherichia coli.

Expression Vectors:

→ Suitable for expressing foreign proteins.

→ Consists of signals necessary for transcription and translation of proteins.

→ Helps to produce large amounts of protein . EG PUC19.

More vectors to know:

Cosmid:

→ contains 'COS' - Cohesive terminus.

→ hybrid vectors from lambda phage DNA and a bacterial plasmid.

Bacteriophage Vectors:

→ Commonly used E.coliphages are 1 phage, M13 phage.

→ More efficient than plasmid DNA.

Lambda Genome: 485026P long [49kb and has 50 genes].

Lambda phage infects E.coli.

Phagemid Vectors:

→ Reconstructed plasmid vectors contain ori gene.

Eg: p Blue script SK (+/-)

Bacterial Artificial Chromosome (BAC) vector:

→ Shuttle plasmid vector.

→ for Cloning large sized foreign DNA.

→ most useful cloning vector in r-DNA technology.

→ Stable, User friendly.

→ can clone DNA inserts of upto 300 kb.

Yeast artificial chromosome(YAC) Vector:

→ occurs in two forms (circular, linear).

→ Circular - multiplies in Bacteria.

→ linear - multiplies in Yeast.

Shuttle Vectors:

→ Designed to replicate in cells of 2 different species.

→ can propagate in one host and move into another without any extra manipulation.

→ Eukaryotic vectors are shuttle vectors.

Competent Host (for transformation with Recombinant DNA)

→ E.cli is the most widely used organism as its genetic make up has been extensively studied.

→ easy to handle, grow can accept vectors safety.

→ E.clo cells divide every 20 minutes.

→ DNA is hydrophilic, cannot pass trough cell membrane.

→ Bacterial cells are forced to take up plasmid by treating with divalent caution such as Calcium.

→ r-DNA can then be forced into cells by placing on ice, briefly at 42°C cheat shock) then back on ice.

→ Enables bacteria to capture r-DNA.

→ For the expression of eukaryotic proteins eukaryotic cells are preferred which is not possible by prokaryotic cell (E.coli).

Methods of Gene transfer:

→r-DNA → Introduce in to host cell.

→ Gene of Interest flanked by controlling sequences (promoter, terminator).

Kinds of Gene transfer

Direct (Vector less)	Indirect or (Vector mediated)
→Chemical mediated Polyethylene glycol Dextran sulphate	→ Agro bacterium tumiefaciens has large plasmid (Ti) and T-DNA
→Micro injection DNA is injected into the nucleus using micro pipette.	→ Natural genetic engineer of Plants → Bt gene for insect resistance
→Electro poration High voltage to protoplast creates pores in plasma membrane which uptake DNA	→ npt III confers resistance to antibiotic Kanamycin are closed in the T-DNA

Liposome Mediated

→ Phospholipid vesicles.

→ DNA is transferred from liposome into vacuole, protects from acidic P^H

→ Lipofection→ Liposome and Tonoplast fusion resulted in gene transfer.

Biolistics

→Foreign DNA is Coated on to minute gold or tungsten bombarded on to target cells.

[Gene gun/ micro projectile]

Screening for Recombinants:

→ After introduction of r-DNA it is essential to identify whether the host cell have received the r-DNA. This process is called screening.

→ Non recombinants do not express the traits.

→Recombinants expresses the traits.

→ Some Methods is Blue -White Selection method.

Insertional Inactivation- Blue white Colony Selection method

→ Reporter gene lacz is inserted in the vector.

→ lacz codes - B galactosidase breaks X gal (5-bromo-4-chloco indolyl-B-D-galacto-pyranoside) into blue coloured product.

→ IF foreign gene is inserted into lac Z if will be inactivated, no blue colour will be develop.(white)

→Non recombinant DNA → Blue coloured colonies.

Recombinants → white coloured colonies.

Antibiotic Resistant Markers:

→ It is a gene that produces protein resistant to an antibiotic

→Transformed DNA can be identified by growing on a medium containing an antibiotic.[Tetra cycline,ampicilli]

→ Recombinants will grow on these medium as they contain antibiotic resistant gene.others may not be able to grow.

Replica plating technique:

Colonies on a culture plate

↓ (Filter plate is pressed)

Copied to second sterile culture plate.

↓

New plate infected with cell in same positions

↓

Second plate is differ from first[may I include antibiotic without growth factor]

↓

Selection of Transformed cells

Molecular techniques-

Isolation of genetic material and gel electro pharoses

→ Used to separate biomolecules.

Principles:

→ Applying (DC) the molecules migrate, based on the charges.

+Ve Charged Cations will move towards -Ve Cathode

-Ve Charged Anions will move towards +Ve Anode

Agarose gel electro phosesis:

→ Purification, separation of DNA fragments ranging in size from few hundred to 20000 base pairs.

→ Poly acrylamide is preferred for purification of smaller DNA fragments.

→ DNA is -vely charged mole wile under an electric field migrates through the gel.

→ Fragments of known size allows size. determination of unknown DNA.

Advantages: Readily detection of DNA bands.

→ Bands of DNA are stained with ethidium bhomide can be visible in UV light will give Orange fluorescence.

ELISA→(Enzyme Linked Immuno Sorbent Assay)

→ Diagnostic tool for Identification of pathogen by using antibodies.

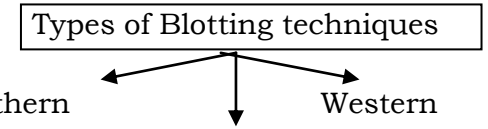
DNA probes→ Popular Tools for identification of viruses and other pathogens.

Nucleic acid hybridization Blotting techniques:

→Analytical tool for the identification of desired DNA or RNA fragments.

→Blotting refers to the process of Immobilization of sample nucleic acids on solid support(nitro cellulose or nylon membrane)

→The blotted nucleic acids are used as target in the hybridization experiments.



Transfer of DNA from agarose to nitro cellulose membrane

Transfer of RNA to nitro cellulose membrane

Transfer of protein to membrane

Southern Blotting techniques-DNA

→Transfer of denatured DNA from Agarose gel to Nitro cellulose Blotting.

→ Introduced by Southern (1975).

Steps:

1. Transfer of DNA from agarose to Nitro cellulose by capillary action.
2. A buffer SSC-Sodium Saline Citrate is used which makes DNA soluble.
3. SS DNA becomes Trapped.
4. DNA is hybridized with a nucleic acid, detected by autoradiography.

Autoradiography:

Captures Image formed in a photographic emulsion due to emission of light.

Northern Blot:

→ RNA is not binding to cellulose nitrate.

→ Alwin etal(1979) Devised a procedure of transferring RNA from agarose into Aitro cellulose filter paper.

→ Amino Benzyl oxy methyl paper which can be prepared from what man 540 paper is used.

Western Blot:

→ Transfer of proteins to Nitrocellulose filter paper.

→ Proteins are identified by probing with a radio labeled antibody.

Difference between Blotting Techniques:

Name	Southern Blotting	Northern Blotting	Western Blotting
Separation of	DNA	RNA	Protein
Denaturation	Needed	Not Needed	Needed
Me membrane	Nitro Cellulose	Amino benzy loxy methyl	Nitro cellulose
Hybridisation	DNA-DNA	RNA-DNA	Protein antibody
Visualising	Auto radio gram	Auto radio gram	Dark room

Bio assay for Target gene effect:

→ Gene Targeting experiments have been targeting the nuclei leads to “gene-knock out”.

Insertion vectors

→ Entirely inverted into target locus

→ Initially circular but during insertion vector become linear

→ Leads to duplication of sequences

Replacement Vectors

→ has Homology region, collinear

→Linearized prior to transfection

→Crossing over occurs to replace the endo geneous DNA.

Transfect ion: Introduction of foreign nucleic acids into cells by non viral methods.

Genome Sequencing: The location of genes on the entire diploid chromosome of an organism.

Genome sequencing and plant genome projects:

Genome: Whole complement of gene that determine all characteristic of an organism.

→ Genome may be nuclear, mitochondrial or plastid genome.

→ Contain both functional and non expressive DNA proteins.

→ Genome project refers to the whole genome of plant is analysed.
→ Genome project have been undertaken in Chlamydomonas, Arabidopsis thaliana, rice and maize.
→ Genome content is expressed in base pairs, content of DNA is expressed in C-value.

Barcode:

Identify the taxon based on its genetic makeup which describes about the characters of any plants.
Evolutionary pattern assessed using DNA:

→ Cladogram will show the genetic distance between 2 taxce.
→ Also showed antiquity or modernity of any taxon with respect to one another.

Genome editing and CRISPR cas 9:

→ has the ability to change an organism DNA.
→ Allow genetic material to be added, removed or altered.
→ Clustered regularly interspaced short palindromic repeats Associated Protein9.
→It is faster, cheaper, more efficient and accurate gene editing method.
→Rice was among the first plants to be used to demonstrate the feasibility of CRISPR. mediated targeted mutagenesis.
→ Imitiyaz khand and Venkatesan sudaresan and colleagues reported one can re-engineer rice to switch it from a sexual to an asexual mode.

RNA Interference (RNAi)

→ Expression of gene involves transcription and translation.
→ Transcription refers to the copying of genetic information from one strand of the DNA (sense strand) by RNA.
→ RNA has to be edited which undertake the process of translation.
→Introms are removed from RNA.
→ Certain regions of DNA are silence.
→ RNA interference is RNA inhibit gene expression by neutralizing targeted m-RNA.
→ RNA is seen in plant feeding nematodes.

Simplified model for the RNA i pathway is based on 2 steps

First step	second step
Trigger RNA is processed in to SIRNA by RNase II enzymes [Dicer and Drosha]	SIRNA are loaded into the effector complies RISC (RNA induced silencing complex) →SIRNA is unwound during RISC assembly. →SSRNA hybridizes with m RNA target

Transgenic plants/Genetically modified crops (GM crops)

Herbicide Tolerant- Glyphosate:

→ Glyphosate herbicide is produced by monasanto USA company [Trade name roundup]
→ Blocks 5-enopyruvate shikimate-3 phosphate synthase (EPSPS) →enzyme involved in biosynthesis of amino acids, vitamins
→ Kill plants

Protocol for Glyphosate tolerant potato plant

Introduction of 'bar' gene
↓
Potato with bar gene
↓
Herbicide Tolerant potato cells
↓
Invitro culture
↓
Organogenesis
↓
Development of Herbicide tolerant plants

Advantages of Herbicide Tolerant crops:

→Higher crop yields.
→ Reduces spray of herbicides
→ Reduces competition between crop and weed.
→ conserve spil microbes
→ Use of low toxic compounds not remain active in soil.

Herbicide tolerant- Basta

→ Contains chemical compound Phosphinothricin.

→Herbicide tolerant gene PPT (L-phosphinothricin) was isolated from Medicago sativa, inhibits glutamine synthase involved in ammonia assimilation.

→ PPT gene was introduced to tobacco was resistant to PPT.

→PAT C phosphinothricin acetyltransferate encodes by bar gene isolated from Streptomyces hygroscopicus introduced to potato, sugar beet.

Insect resistance - Bt crops:

→ Bt Cotton → GMO, produces an insecticide activity to bollworm.

→ Bacillus thuringiensics produce 200 different Bt toxins.

→ Insecticidal to larvae of moths of beetle, butter flies and bollworms.

→ Genes encoded for toxic crystal cry group of endo toxin.

→ cry toxins dissolves in the gut of the insects, block certain vital nutrients, regulation of potassium ions are lost, results in death of epithelid cells in the intestine leads to death of the larvae.

Advantages:

→High yield

→Reduction in Insecticide usage.

→Reduction in the cost of cultivation.

→Control bollworms

Disadvantages:

→Cost of seed is high.

→Effectiveness up to 120 days.

→Ineffective against aphids, whitefly.

→Affect pollinating insects.

Bt Brinjal:

→Inserting a crystal protein gene (cryl Ac) from Bacillus thuringiensis into the genome of Brinjal.

→Promoters, terminators and antibiotic resistance marker gene into the brinjal plant is accomplished using Agro bacterium.

→Bt Brinjal is resistant against Lepidopteron insects, Leucinodes orbonalis.

iii) Dhara mustard Hybrid (DMH)

DMH II → Developed by a team of Scientists centre for Genetic manipulation of crop plants at Delhi university.

→ It is HT mustard (Herbicide Tolerant).

→ Genetic modification by adding genes from soil bacterium.

→ DMH-11 contains 3 genes.[Bar gene, Barnase and Barstar from soil bacterium]

Virus Resistance:

→ Biotechnological intervention is used to introduce viral resistant genes into the host plant.

→Introducing genes that produce resistant enzymes which can deactivate viral DNA.

Flavr savr Tomato:

→Agrobacterium medicated genetic engineering technique.

→Ripening process of tomato is slowed down, preventing softening resistant to rotting.

→ Introducing an antisense gene which interferes with the production of polygalacturonase, helps in delaying the ripening process.

Golden rice - Bio fortification:

→A variety of oryza sativa is produced through genetic engineering of bio synthesized beta carotene, precursor of vitamin A

→Developed by Ingo potrykecs and his group.

→ Addition of 3 bata carotene biosynthesis genes. namely psy (Phytoene synthase) from Narcissus pseudo narcissus (Daffodil) Crt-1 gene from Erwinia auredorora-soil bacterium and lyc (lycopene cyclase) gene from wild type rice endosperm.

→Golden rice can control childhood blindness Xerophthalmia

GM foods - Benefits:

→ High yield

→ 70% reduction of pesticide usage

→ Reduce soil pollution.
→ Conserve soil microbes

Risks:

→Affect lives, kidney function and cancer.
→Hormonal Imbalance.
→Anaphylactic shock.
→Adverse effect in Immune system.
→ Loss of viability of seeds.

PHB (Poly hydroxyl butyrate)

→ PHP (Polu hydroxyl alkanoates) degradable polymer, thermoplastics, bio compatible

PHB → Several medical applications – Drug delivery, Scaffold and heart valves

→ PHAs produced by G^{+ve} bacteria such as Bacillus megaterium, B.subtilis Cornyne bacterium glutamicum, G^{-ve} bacteria like pseudomonas, Alcaligenes eutrophus.

PLA (Poly lactic acid)

→ Bio degradable, bio active, Thermoplastic.

→ Aliphatic polyester derived from corn, starch, camava root or sugarcane.

→ For PLA production→2 monomees are used: lactic acid and cyclic diester, lactide.

→ polymerization of Lactide with metal catalysts like tin octoate insolution.

→The metal catalyzed reaction results in PLA.

GFP: Green fluorescent protein

→ Contains 238 amino acid residues of 26.9 KDa. exhibits bright green inflorescence when exposed to UV (395nm).

→First Isolated from Jelly fish Aequorea Victoria.
→Excellent tool in biology.
→Its ability to form internal chromophase without requiring accessory Co-factors; enzymes, other than molecular O_2.
→GFP is used as a reporter of expression.
→ used to make biosensors.

Protocol

GFP
↓
Isolated from Aequorea Victoria
↓
Gene Splicing
↓
Introduced in to Arabidopsis thaliana
↓
GFP expressed in A-thaliana plant

Biopharming (molecular pharming)

→ Use of transgenic plants genetically engineered to produce pharmaceutical substances.
→ Use of plant systems as bioreactors.
Eg: Goldenrice.

Bioremediation:

→use of microorganisms or plants to clean up environmental pollution.
→ used to treat waste water, industrial waste, Solid waste, heavy metals, petro chemical residues.
→ Less expensive, eco friendly.

Strategies for bioremediation in soil and water can be as follows:

→ Use of Indigeneous microbes as indicators.
→ Designed microbial inoculants.
→ Use of plants for bioremediation green technology.

Some examples of bioremediation technologies are:
Phytoremediation: use of plants to treat environmental pollutants.
Mycoremediation: use of fungi to bring about remediation of environmental pollutants.

Bioventing: Increases the O_2 to accelerate the degradation of environmental pollutants.

Bioaugmentation: Addition of microbes to speed up degradation process.

Composing: Solid waste is converted into manure by microbes.

Rhizofiltsation: Uptake of metals by Rhizosphere microbes.

Rhizostimulation: Stimulation of plant growth by reducing toxic materials, providing better growth condition.

Limitations of Bioremediation:

→ Only biodegradable contaminants can be transformed

→ Must be made in accordance to the conditions at the contaminated site

→ Small scale test on a pilot scale must be performed

→ Consortium of microbes are created using genetic engineering for bioremediation has great potential

Biofuel: Algal Bio fuel

→ Alternative to liquid fossil fuels

→ Botryococcus brain; is used to produce algal biofuel

→ Alternative to the biofuel obtained from corn and sugarcane

Biological hydrogen production by algae:

→ In normal photosynthesis Chalamydomonas reinhartii releases O_2

→ When deprivation of sulfur, it produces hydrogen

→ Electrons are transported to fessedoxins Fe-hydrogenase enzymes. Combine them into the production of hydrogen gas

Biopropecting:

→ Discovery and commercialization of new products from bio-resources

→ Involve Biopiracy, indigenous knowledge of nature originating with indigenous people is used by others for profit without authorization or compensation to the Indigenous people

Biopiracy:

→ Manipulation of Intellectual property rights laws to control over national genetic resources without giving adequate remuneration or recognition to the original possessors.

Eg: Recent parents granted by the U.S parent and Trade marks office to American Companies on turmeric neem, basmati

Bio piracy of neem:

→ Neem oil control fungal and bacterial infections

→ USDA (United States Department of Agriculture) pirating the knowledge

→ W.R. Grace in early 90's sought a patent on method for controlling of diseases on plants by the aid of extracted hydrophobic neem oil

Biopiracy of Turmeric:

→ United states patent and trademark office in the year 1995 granted patent to the method of use off turmeric as an antiseptic agent

→ Turmeric has been used by the Indians as a home remedy for the healings if wounds, rashes

→ In the journal article by Indian medical association (1953), remedy was mentioned

→ Its not a new invention, a part of the traditional knowledge of the Indians.

Bio Piracy of Basmati:

→ US parent trademarks office (1997) granted patent on basmati rice lines

→ Patent covers the process of breeding Rice Tec's novel rice lines, starch cotent, cooking properties

→ India has periled the United states take the mater to the WTO

→ Due to few decisions US parent office, Ricetec had no choice choice but to lose most of the claims, most importantly the right to call the rice "Basmati"

→ In the year 2002 the final decision was taken

→ Ricetec dropped down 15 claims

→ The parent office ordered the parent name to be changed to "Rice Lines 867"

Application of Biotechnology:

→ Enables us to find the beneficial way of life

→ Wide applications in agriculture medicine, environment, industries

→ Production of Transgenic varieties (Bt-Cotton), rice, tomato, potato

→ Development of transgenics as pesticide, stress, disease resistants

→ Human Insulin synthesis [E.coli]

→ Synthesis of vaccines, antibiotics, beverages

→ Biochip, Bioremediation, Phytoremediation

→ Aseptic cultivation of totipotent plant cell into plant clones

→ Single cell protein from spirulina

→ Production of secondary metabolities

→ Production of bio fertilizer, enzymes bio fuel