BIOTECHNOLOGY PRINCIPLES AND PROCESSES

- Biotechnology deals with the techniques of using living organisms or enzymes from organisms to produce products useful to humans.

- The term 'Biotechnology' was given by Karl Ereky (1919)

- According to European federation of biotechnology (EFB) biotechnology is the integration of natural science and organism, cells, parts there of, and molecular analogues for products and services.

Principles of Biotechnology

The two core techniques developed modern technology are -

i. Genetic Engineering → Which is modification of chemical nature of DNA / RNA and their introduction into another host organism to change the phenotypic characters of the host.

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ii. Sterilisation Method - Sterilisation method to maintain growth and manipulation of only the desired microbes or cells in large quantities, for the manufacture of biotechnology products like - antibiotics, Vaccines, enzymes etc.

The basic steps in Genetic engineering include (GMO)

i. Identification of DNA with desirable Genes.

ii. Introduction of the DNA into host to form recombination DNA (rDNA)

iii. Maintenance of DNA in host and gene cloning.

★ In 1972, Stanley cohen and Herbert boyer Constructed the first recombinant DNA.

Steps Carried out in Constructing first recombination DNA

i. A gene encoding antibiotic resistance in the native plasmid of Salmonella typhi murium was identified, plasmid is an autonomously replicating circular extra-chromosomal DNA.

ii. The desired DNA was cut at specific locations by restriction enzymes.

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iii The cut DNA was linked to plasmid DNA and transferred to E. coli for Gene multiplication

Tools of Recombinal DNA Technology?

i Enzymes → Various enzymes involved in Genetic engineering. (like — Restriction enzyme, ligases DNA, Alkaline Phosphatase & DNA Polymerases

ii Cloning vector → Plasmids and bacteriophages have the ability to replicate within bacterial cells independent of the control of chromosomal DNA.

iii. Competent host → The host should be competant enough to take up the foreign DNA.

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Enzymes

1. Restriction Enzymes

- The restriction enzymes are called "Molecular Scissors" and are responsible for cutting DNA.

- They are present in bacteria to provide a type of defence mechanism called restriction modification system.

- ★ 1st restriction endonuclease Hind II was isolated by Smith wilcox and Kelley in 1968. (recoganising a specific sequence of 6 base pair called recognition sequence)

* THE CONVENTION FOR NAMING RESTRICTION ENZYMES

- The first letter of the name comes from the Gene

- The second two letter come from the species of the prokaryotic cell from which they were isolated e.g EcoRI comes from E. Coli RY13

- In EcoRI, the letter 'R' is derived from the name of strain

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- Roman numbers following the names indicate the order in which the enzymes were isolated from that strain of bacteria

- 900 restriction enzymes that have been isolated from over 230 strains of bacteria.

These are of two kinds (Restriction enzyme)

1. Exonucleases
2. Endonucleases

- Exonucleases remove nuclotides from the end of the DNA whereas, endonucleases makes cuts at specific position within the DNA.

- ~~Each~~

Mechanism of Action of Restriction Endonucleases

- functions by 'inspecting' the length of a DNA Sequence.

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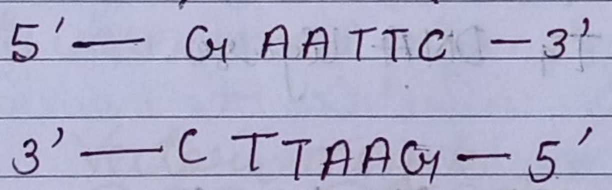
- once it finds specific recognition sequence, it will bind to DNA

- Cut each of the two strands of double helix at specific points in their sugar phosphate backbones.

- Each restriction endonuclease recognizes a specific palindromic nucleotide sequence in the DNA.

- The palindrome in DNA is a sequence of base pair that reads same on the two strands when orientation of reading is kept the same.



- e.g. the following sequences read the same on the two strands in 5' → 3' direction, this is also true if reads in the 3' → 5' direction.

- NOTE → Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites, but b/w the same two bases on the opposite strands which leaves a single stranded portions at the ends and the overhanging stretches called Stickyends on each strand.

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- When cut by the same restriction enzyme (vector & source DNA), the resultant DNA fragments have the same kind of 'sticky-ends' and these can be joined together using DNA

2. DNA Ligases (Molecular Glue)

- This enzymes repair broken DNA by joining two nucleotides.

- Used in genetic engineering to reverse the action of restriction enzyme.

- e.g → T_4 DNA Ligase.

3. Alkaline Phosphatase (AP)

- Remove phosphate group from the 5' end of a DNA molecule.

- leaving it free 5' hydroxyl group.

- Prevent unwanted self-ligation of vector DNA molecules during formation of recombinant DNA.

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4- DNA Polymerases

- This enzyme helps in in Vitro synthesis of Complementary DNA (cDNA) Strand on DNA templates.

Cloning Vectors

- A cloning vector is a small piece of DNA, taken from any organism into which a foreign DNA fragment can be inserted for cloning purpose.

- Plasmids and bacteriophages have the ability to replicate within bacterial cells independent of the control of chromosomal DNA.

Vectors may be

① Plasmid :- These are autonomously replicating circular extra-chromosomal DNA. (occur- Yeasts & Bacteria)

② Bacteriophages :- These are viruses infecting bacteria.

★ The best known vector is the Plasmid vector

pBR322 is the first artificial cloning vector developed in 1977.

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by Boliver and Rodriguez from E. coli plasmid.

★ The following are the features that are required to facilitate cloning into a vector are :-

1. Origin of replication (ori)
2. Selectable maker
3. Cloning sites

• Origin of replication (ori)

- This is the sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cell.

- It also controls the copy no. of the linked DNA

• Selectable Marker

- It helps to select the host cells which contain the vector (transformants) and eliminate the non-transformants.

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- Transformation is defined as the procedure by which a piece of DNA is introduced into a bacterial host.

- The genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or Kanamycin etc are useful selectable markers for E. coli as the normal E. coli cells do not carry resistance against any of these antibiotics.

- The normal E. coli cells do not carry resistance against any of these antibiotics.

Cloning Sites

- To link the alien DNA, the vectors require very few (mostly single) recognition sites for the restriction enzymes.

- More than one recognition sites within the vector can complicate the gene cloning as it will generate several fragments.

- Ligation of alien DNA can be carried out a restriction site present in one of the two antibiotic resistance genes.

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• Vectors for Cloning genes in plant and animals

Plant

- Vector for cloning genes in plants is Agrobacterium tumifaciens, a pathogen of several dicot plants which delivers a piece of DNA known as 'T-DNA' to transforms normal plant cell into a tumor and direct these tumor cells produce the chemicals required by the pathogen.

- The tumor inducing (Ti) plasmid of Agrobacterium tumifaciens has now been modified into a Cloning Vector tumour formation by Agrobacterium tumifaciens

Animal

- Vector for cloning genes in animals is retrovirus which transforms normal cells into Cancerous cells.

- Retroviruses have been disarmed and used to deliver desirable gene.

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★ Selection of recombinant formed can be done by one of the following methods:-

(a) Inactivation of antibiotics

- If a foreign DNA ligates at the Bam HI site of tetracycline resistance gene in the vector pBR322, the recombinant plasmid loses the tetracycline resistance due to insertion of foreign DNA
- It can still be selected out from non-recombinant ones by plating the transformation on ampicillin ~~Containg~~ Containing tetracyline
- The transformation growing on ampicillin containing medium are then transferred on to a medium containing tetracyline.
- The recombination can grow in ampicillin containg medium but not on the containing tetracyline whereas non-recombinants can grow on the medium containing both antibiotics and thus recombinants are selected.

(b) Insertional Inactivation

- On the basis of colour production in the presence of Chromogenic substrate the recombinants

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and non recombinants can also be differentiated)

- Here, a recombinant DNA is inserted within the coding sequence of an enzyme β-Galactosidase which results into inactivation of the enzymes.

Reporter enzyme → Produced by Reporter gene.

- Bacterial Colonies having inserted plasmid shows no colouration

- While those without plasmid show blue colour.

Competent Host (For Transformation with Recombinant DNA)

- DNA being a hydrophilic molecule cannot pass through cell membranes.
- Therefore the bacteria should be made competent to accept the DNA molecules.

- Competency is the ability of a cell to take up foreign DNA.

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The cell is made Competent by the following method:

(a) chemical method
(b) Physical method
(c) Disarmed pathogen vector

☆ (a) Chemical Methods

- The cell is treated with specific concentration of a divalent cation as Calcium to increase pore size in cell wall.

- The cell are incubated with recombinant DNA on ice followed by placing them briely at 42°C and then putting it back on ice. This is called heat shock treatment.

- The bacteria now take up the recombinant DNA.

☆ (b) Physical Methods

• The physical method include.

• Micro- injection method - Recombinant DNA is directly injected into the nucleus of an animal cell.

• Biolistic or Gene gun method - cell are bambared with high Velocity micro particle of

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gold or tungsten coted with DNA in plant.

★ (a) Disarmed pathogen vector

- use to transfer DNA.

- Vectors that cause infection are used to infect the cell to transfer the recombinant DNA into the ~~cell~~ host.

- retrovirus, papillomavirus & adenovirus in case of Animals.

- Agrobacterium in case of Plant.

PROCESS OF RECOMBINATION DNA TECHNOLOGY

① Isolation of the Genetic Material (DNA)

1) RNA is removed by treatment with ribonuclease and proteins are removed by treatments with Protease

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2) After several treatments, the purified DNA is precipitated by adding chilled chilled ethanol.

3) The bacterial / plant / animal cell is broken by enzymes to release DNA, along with RNA and polysaccharides and lipids

4) Bacterial cell is treated with enzyme lysozyme

5) Plant cell is treated with enzyme cellulose

6) Fungi cell is treated with chitinase

② Cutting of DNA at specific locations

- The DNA is cut using restrictions enzymes.

- The purified DNA is incubated, with the specific enzymes at conditions optimum for the enzyme to act.

③ Isolation of desired DNA fragments

- Using agarose gel electrophoresis the activity of the restriction enzymes can be checked.

- Since the DNA is -vely charged, it moves towards the +ve electrode or anode and in the process, DNA fragments separate out based on their sizes.

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- The desired DNA fragments is eluted out

④ Amplification of gene of interest using PCR

- The polymerase chain reaction (PCR) is a reaction in which amplification of DNA sequence is carried out in vitro

- The technique was developed by Kary Mullis in 1985 and for this he received ★Nobel prize for chemistry in 1933.

Requirement of PCR

1. DNA template → The double stranded DNA that needs to be amplified

★ 2. Primers → Small chemically synthesized ★oligonucleotide (short segment of DNA) of about 10-18 nucleotids that are complementary to a region complementary to a region of template DNA.

3. Enzymes → Two commonly used enzymes are Taq Polymerase (isolated from thermophilic bacterium, thermus aquaticus

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and Vent polymerase (Isolated from thermococcus litoralis)

4. Nucleotide Bases

These are added by DNA polymerase to the growing chain.

PCR is carried out in the following three steps

Step I → Denaturation ⇒

- The double stranded DNA is denatured by subjecting it to high temperature of 95°C for 15 second

- Each separated single stranded strand now acts as template for DNA Synthesis.

Step II → Annealing ⇒

- Two sets of primers are added which anneal to the 3′ end of each separated strand.

- Primers act as initiation of replication.

Step III → Extension ⇒

- DNA Polymerase extends the primers by adding nucleotides Complementary to the template

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provide in the reaction).

- A thermostable DNA polymerase (Taq polymerase) is used in the reaction which can tolerate the high temp of the reaction.

- All these steps are repeated many times to obtain several copies of desired DNA.

⑤ Ligation of DNA fragments into a Vector.

- The vector DNA and source DNA are cut with the same endonuclease to obtain sticky ends.

- These are then ligated by mixing vector DNA gene of interest and enzyme DNA ligase to form a recombinant DNA.

⑥ Insertion of Recombinant DNA into the host cell / organism.

- Introduction of ligated DNA into recipient cells occur by several methods, before which the recipient cells are made competent to receive the DNA.

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- If recombinant DNA carrying antibiotic resistance (e.g. ampicillin) is transferred into E. coli cells, the host cell is transformed into ampicillin-resistant cells.

- On growing transformed cells on agar plates containing ampicillin, only transformants will grow and others will die.

⑦ Culturing the host cells

- The transformed host cells are grown in appropriate nutrient medium in optimal conditions.

- The DNA gets multiplied and expresses itself to to form desired product.

⑧ Extraction of desired gene product

- When a protein encoding gene is expressed in a heterologous host, it is called a recombinant protein.

- The cells having genes of interest can be grown on a small scale or on a large scale.

- On small scale, the cells are grown on culture in laboratory and then the expressed protein is extracted and purified by different separation

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is extracted and purified by different separation techniques.

- On large scale the cells are grown in a Continuous Culture system in which fresh medium is added from one side to medium cells in exponential growth phase and desired protein is collected from the other side.

- In large scale method, larger biomass is produced which lads to high yield.

BIOREACTORS

- ~~Block~~ Bioreactors are vessels of large Volume (100-1000 litres) in which raw materials are biologically converted into specific products.

- It provided all the optimal conditions for achieving the desired product by providing optimal Growth Conditions like- temperature, pH, Substrate, Salt, vitamins, and oxygen.

- Stirred-tank bioreactors are Commonly used bioreactors.

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- These are cylindrical with curved base to facilitate proper mixing of the contents. (Single Stirred-tank bioreactor)

Bioreacter Components

- 1) An agitator System
2) An oxygen delivery System
3) Foam control System
4) Temperature Control System
5) pH Control System
6) Sampling ports to withdraw cultures periodically.

- The Stirrer mixes the contents and make oxygen available throughout the bioreacter.

- Sparged Stirred tank reactor is a stirred type reactor in which air is bubbled.

DOWNSTREAM PROCESSING

- All the processes to which a product is subjected to before being marketed as a finished product are called Downstream processing.

It includes

- (a) Separation of the product from the reactor.
- (b) Purification of the product
- (c) Formulation of the product with suitable preservatives
- (d) Quality control testing and clinical trials in case of drugs.

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