Strain Improvement:

After an organism producing a valuable product is identified, it becomes necessary to increase the product yield from fermentation to minimize production costs.

How the yield can be increased? Product yields can be increased by:

- Developing a suitable medium for fermentation
- Refining the fermentation process
- Improving the strain for higher production.

Generally, major improvements arise from the last approach; therefore, all fermentation enterprises place a considerable emphasis on this activity (i.e the potential productivity of the organisms is controlled by its genes and hence their genome must be altered for the maximum production of desired product).

What is strain improvement?

The techniques and approaches used to genetically modify strains, to increase the production of the desired product are called strain improvement or strain development (genetically modify strain to increase the production of the desired product).

Characteristics of strain improvement:

- Which shows rapid growth.
- Which has large cell size, for easy removal from the culture fluid.
- Which require shorter fermentation times.
- Which do not produce undesirable pigments.
- Which have reduced oxygen needs.
- Which exhibit decreased foaming during fermentation.
- Which are able to metabolize inexpensive substrates.
- Do not show catabolite depression.
- Which shows Genetic stability.
- Which are non-toxic to humans.

Aims of Strain Improvement:

- Increasing productivity.
- Genetic stability.
- Reduction of cultivation cost.
  - – Lower price in nutrient
  - – Lower requirement for oxygen
- Other Metabolites in the fermentation process

Strain improvement is based on the following three approaches:

I –Mutation:

A **mutation** is any change in the base sequence of DNA (deletion, insertion, inversion, substitution) . One of the most successful approaches for strain improvement. The types include:

A- Spontaneous mutation:

Occur spontaneously at the rate of 10^{-10} and 10^{-15} per generation and per gene.Occur at low frequency and hence not used much in industrial strain improvement.

B- Induced mutation:

The rate of mutation can be increased by various factors and agents called mutagens{any agent (physical or chemical) that can induce a genetic mutation or can increase the rate of mutation}. ionizing radiations (e.g. X-rays, gamma rays) non-ionizing radiations (e.g. ultraviolet radiations) various chemicals (e.g. mustard gas, benzene, ethidium bromide, Nitrosoguanidine-NTG).

C-Site directed mutations(SDM) (site-specific mutagenesis) :

Change in the base sequence of DNA changing the codon (A sequence of three nucleotides which together form a unit of genetic code in a DNA or RNA molecul) in the gene coding for that amino acid. Can be done by protein engineering method desired improvements might be:

*Increased thermo stability.

*Altered substrate range.

*Reduction in negative feedback inhibition.

*Altered pH range.

II- Protoplast fusion: Protoplast has several biological definitions:

A protoplast is a plant, bacterial or fungal cell that had its cell wall completely or partially removed using either mechanical or enzymatic means.

Protoplasts: Have their cell wall entirely removed.

Spheroplast: Have their cell wall only partially removed.

More generally protoplast refers to that unit of biology which is composed of a cell's nucleus and the surrounding protoplasmic materials.

It is widely applied in plant breeding, but is gaining importance in microbes(bacteria &fungi).

In this technique, cells from genetically related or unrelated spp. are fused to produce hybrid cells of desirable traits (Fig.:1) . Protoplasts can be made by degrading cell walls with a mixture of the appropriate polysaccharide degrading enzymes (Tabel:1)

Table(1): Enzymes for the preparation of protoplasts .

Type of cell	Enzyme
Plant cells	Cellulase, pectinase, xylanase
Gram-positive bacteria	Lysozyme (+EDTA)
Fungal cells	Chitinase

Once the cell wall has been removed the resulting protoplasts are spherical in shape , protoplast becomes very sensitive to osmotic stress .This means cell wall digestion and protoplast storage must be done in an isotonic solution to prevent rupture of the plasma membrane. The protoplast that formed are separated from cells & debris by differential centrifugation. The fusion of protoplast is carried out by one of these procedures:

* Sodium nitrate treatment

* High pH or calcium ions treatment

* Polyethylene glycol (PEG) treatment:

In some studies, high PH/Ca++and PEG method have been combined.

* Electrofusion : Recently, mild electrical stimulation is being used to fuse protoplasts.

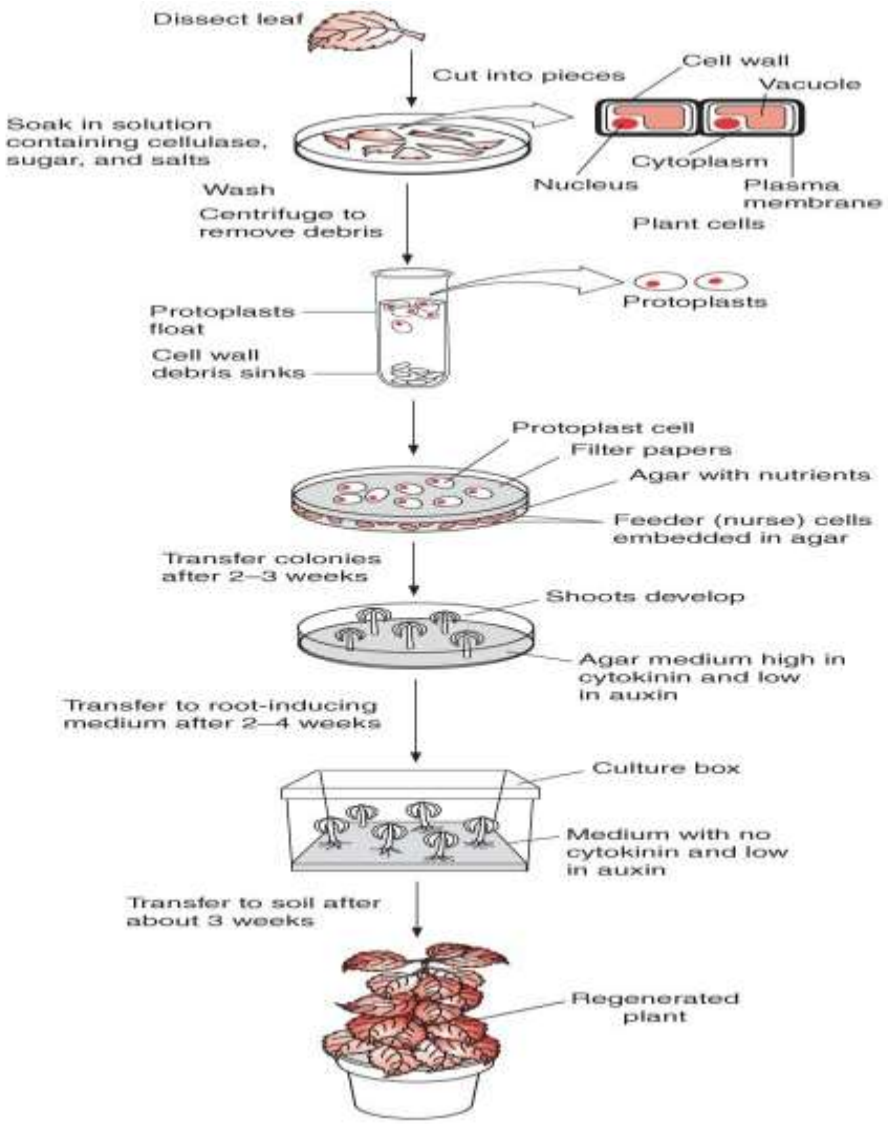


Fig.(1): Protoplast fusion and regeneration of a hybrid plant.

After the fusion process, cell are transferred to a generation medium in which formation of cell wall takes place .Then plating the hybrid on a selective nutrient media which supports the growth of hybrid cells .The final step is the selection of those hybrid cells which are of industrial importance.

Protoplast fusion depends on the following criteria:

- Lytic enzyme.
- Osmotic stability.
- Age of the mycelia.

- Inoculation period.
- Regeneration medium.
- Regeneration frequency .
- PEG concentration.
- Fusant formation.

Types of protoplast fusion:

*Intraspecific hybridization: between different sub-spp within a spp.

* Interspecific hybridization: between different species within the same genus.

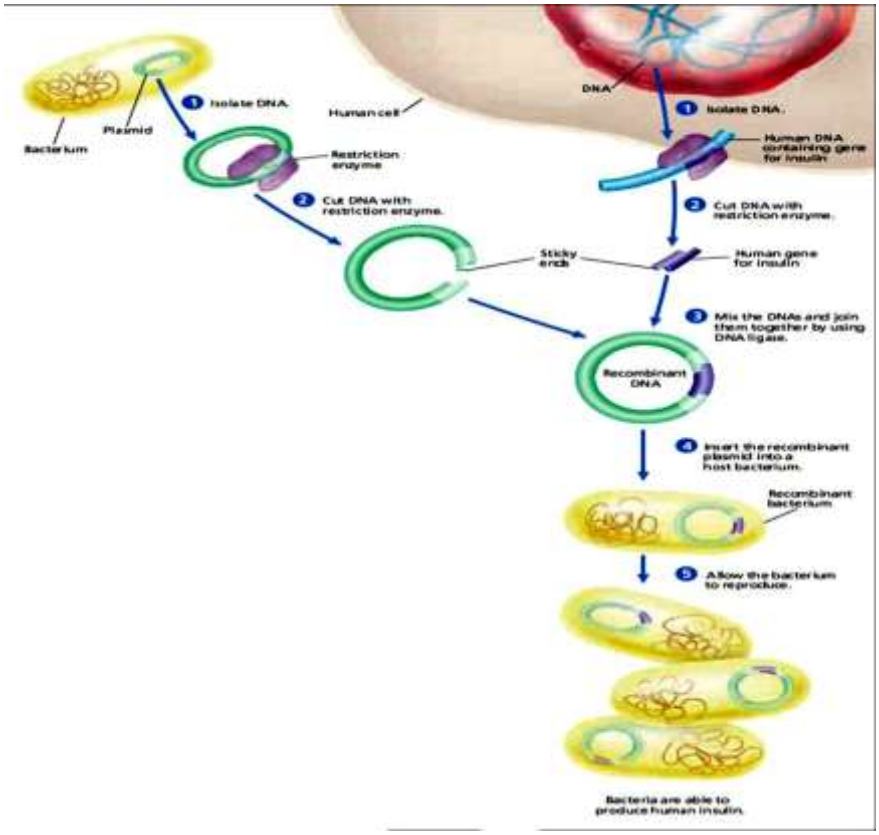
* Intergeneric hybridization : between different genera.

III- Recombinant DNA technology: Which characterized by:

- The more advanced method .
- To increase the yields and consistencies of enzymes.
- Genetic material derived from one species may be incorporated into another where it is expressed.
- Increases the production of heterologous proteins by :
    * Increasing the gene expression using strong promoters.
    *Deletion of unwanted genes from the genome.
    *Manipulation of metabolic pathways.

Steps involved in rDNA process:

- Preparation of desired DNA
- Insertion of desired DNA into vector DNA
- Introduction of recombinant DNAs into host cells
- Identification of recombinants
- Expression of cloned genes.



Bacterial DNA is usually in the form of a single chromosome and plasmids; the latter are autonomous self-replicating accessory pieces of DNA .

Each plasmid carries up to a few hundred additional genes and there may be as many as 1000 copies of a plasmid per cell. They contain supplemental genetic information coding for traits not found in the bacterium's chromosomal DNA.

Unlike most eukaryotic organisms, bacteria have no form of sexual reproduction. However, they are able to exchange some genetic material via the processes of conjugation, transduction and transformation.

***Conjugation**: Involves cell to cell contact or through sex pili and the transfer of plasmids. Conjugation involves a donor cell which contains a particular type of conjugative plasmid, and a recipient cell which does not. The donor strain's plasmid must possess a sex factor as a prerequisite for conjugation; only donor cells produce pili. The sex factor may on occasion transfer part of the hosts' DNA.
***Transduction**: Is the transfer of bacterial DNA from one bacterial cell to another by means of a bacteriophage. In this process a phage attaches to, and lyses, the cell wall of its host. It then injects its DNA (or RNA) into the host.

* **Transformation:** Is a change in genetic property of a bacterium which is brought about when foreign DNA is absorbed by, and integrates with the genome of, the donor cell . Cells in which transformation can occur are 'competent' cells.