Strategies for improvement of industrially important strains

-Dr. Ekta Khare
Department of Microbiology
Institute of Biosciences & Biotechnology,
CSJM University, Kanpur

What are strains?

- A strain is a genetic variant or subtype of a microorganism (e.g., a virus, bacterium or fungus).
- Microbial strains can also be differentiated by their genetic makeup using metagenomic methods to maximize resolution within species.

What are industrial strains?

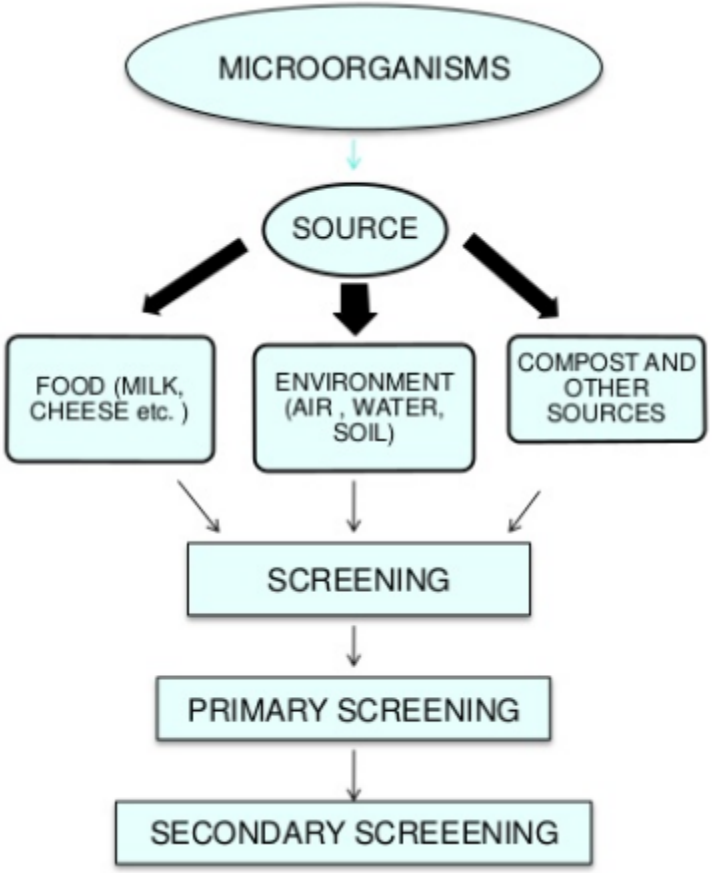
- Strains which synthesize one component as the main product are preferable, since they make possible a simplified process for product recovery.

Why is strain development important in industrial microbes?

- Prerequisite for efficient biotechnological processes at industrial scale is the use of microbial strains which produce high titre of the desired product.
- The process of enhancing the biosynthetic capabilities of microbes to produce desired product in higher quantities is defined as microbial strain improvement.

From where we can find industrial strains?

- The first step in developing producer strains is the **isolation** of concerned microorganisms from the natural habitats.
  - What we are looking?
  - From where we can get?
- The procedure of isolation, detection, and separation of microorganisms of our interest from a mixed population by using highly selective procedures is called **Screening.**



STRAIN IMPROVEMENT

- The development of industrial strains, that can tolerate cultural environment and produces the desired metabolite in large amount from wild type strain is called **strain improvement.**
- The rate of production is controlled by genome of an organism.
- Hence the rate of production can be increased by inducing necessory changes in genome of the organism. Hence it is also called **genetic improvement of microbial strain**.

Proper strain used in industry genetically regarded as safe (GRAS)

Targets of strain improvement

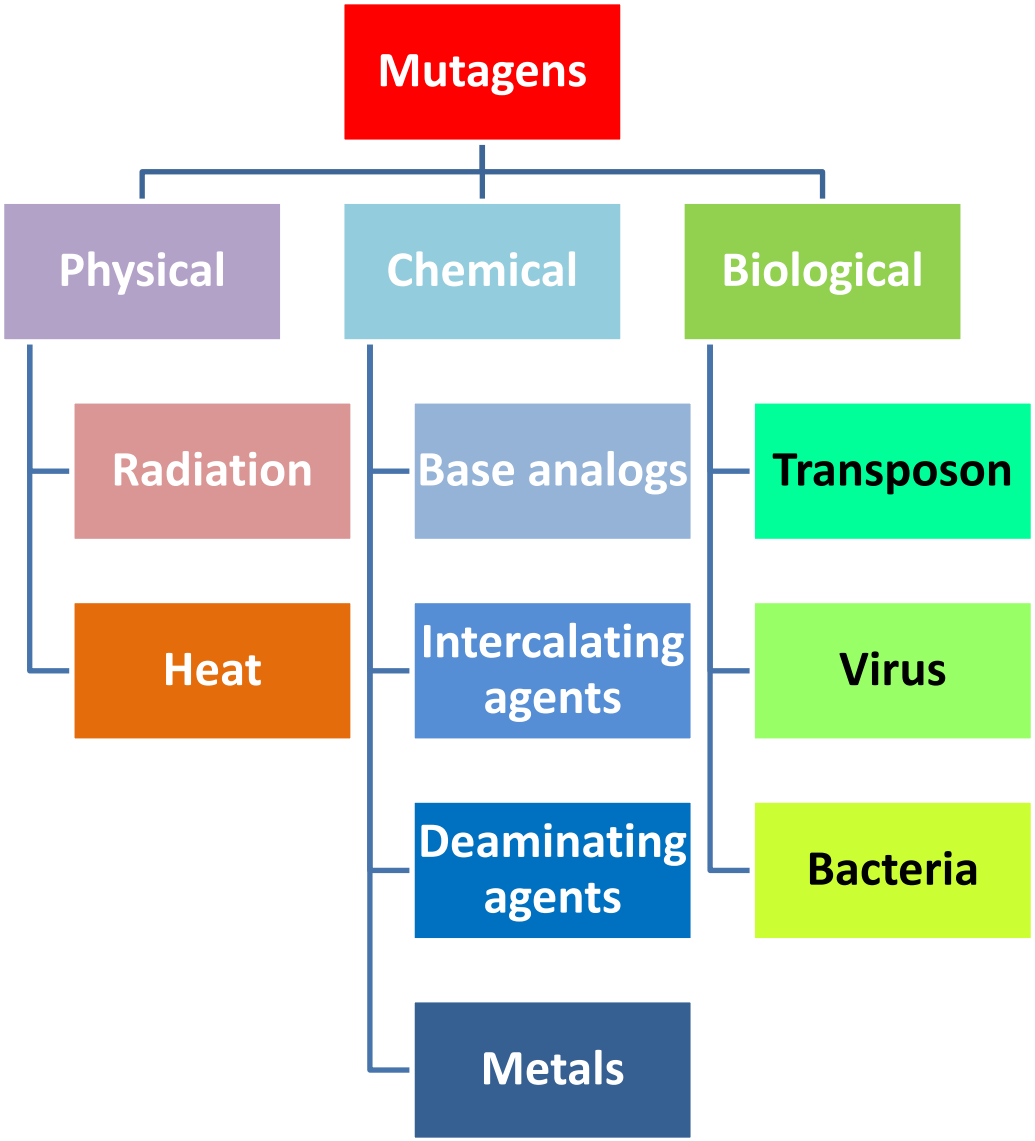
- Rapid growth
- Genetic stability
- Non-toxicity to humans
- Large cell size, for easy removal from the culture fluid
- Ability to use cheaper substrates
- Elimination of the production of compounds that may interfere with downstream processing
- Increase productivity.
- To improve the use of carbon and nitrogen sources.
- Reduction of cultivation cost
  - -lower price in nutrition.
  - -lower requirement for oxygen.
- Production of
  - -additional enzymes.
  - -compounds to inhibit contaminant microorganisms.

Methods of strain improvement

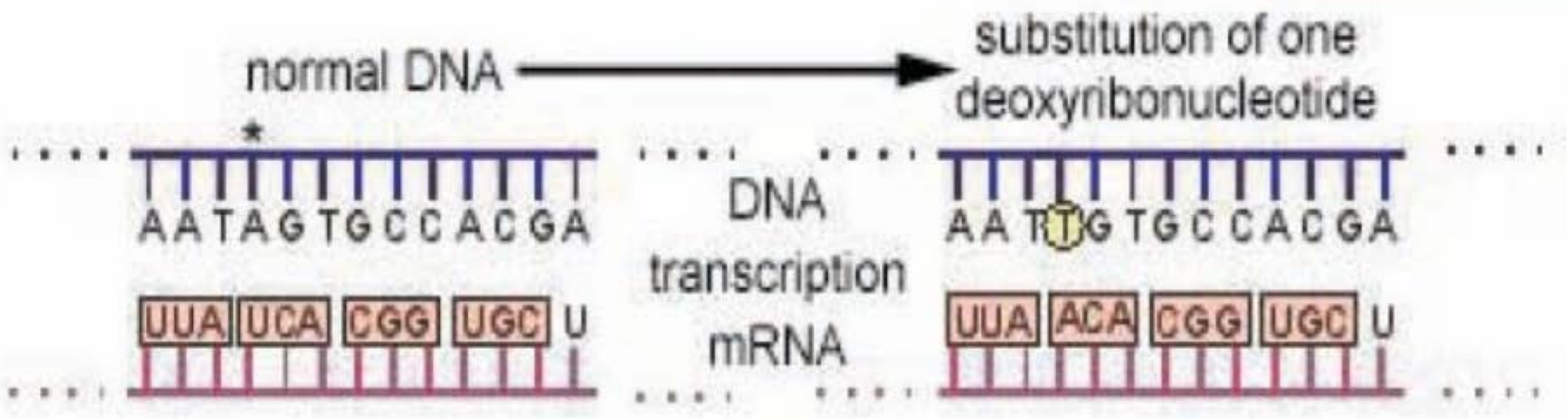
- Mutation and mutant selection
- Recombination
  - Transduction
  - Transformation
  - Conjugation
  - Protoplast fusion
  - Parasexual recombination
- Recombinant DNA technology

Mutation and mutant selection

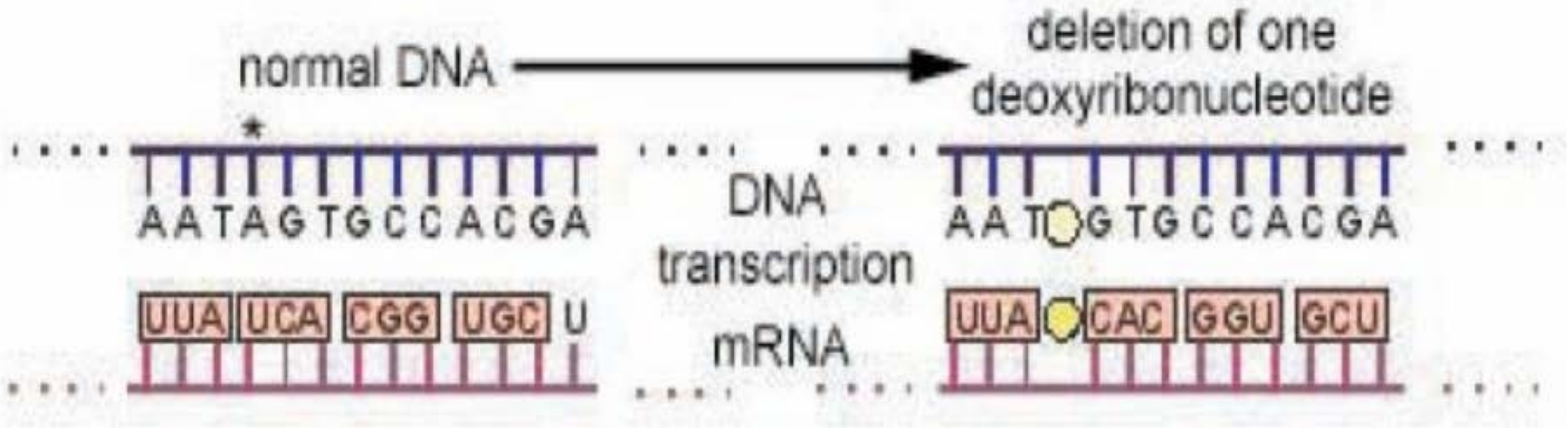
- A mutation is a sudden and heritable change in the traits of an organism.
- Mutations occurring without any specific treatment are called “spontaneous mutation”.
- Mutation are resulting due to a treatment with certain agents are known as “induced mutation”.
- Application of mutagens to induce mutation is called mutagenesis.
- Agents capable to induce mutations are called mutagens.


Mutagens
Physical
Chemical
Biological
Radiation
Heat
Base analogs
Intercalating agents
Deaminating agents
Metals
Transposon
Virus
Bacteria

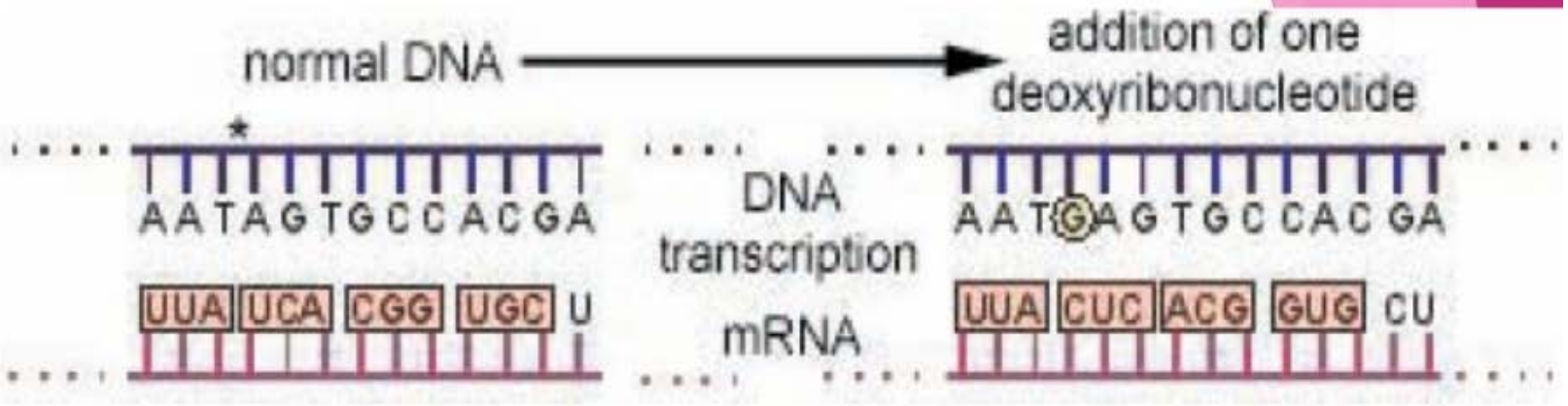
MUTAGEN	MUTATION INDUCED	IMPACT ON DNA	RELATIVE EFFECT
Ionizing Radiations-X Rays,gamma rays	Single or double strand bearkage of DNA	Deletion/structural changes	high
UV rays,chemicals	Pyrimidine dymerisation	Trnsversion,deletion,frameshift transitions from GC → AT	Medium
Hydroxylamine(NH_2OH	Deamination of cytosine	GC A T transitions	low
N-Methyl –N'-Nitro N-Nitrosoguanidine	Methylation of bases and high pH	GC AT transitions	high
Nitrous acid(HNO_2)	Deamination of A,C & G	Bidirectional transitions,deletion,AT GC/GC AT	Medium
Phage,plasmid,DNA transposing	Base substitution,breakage.	Deletion,duplication,insertion.	high



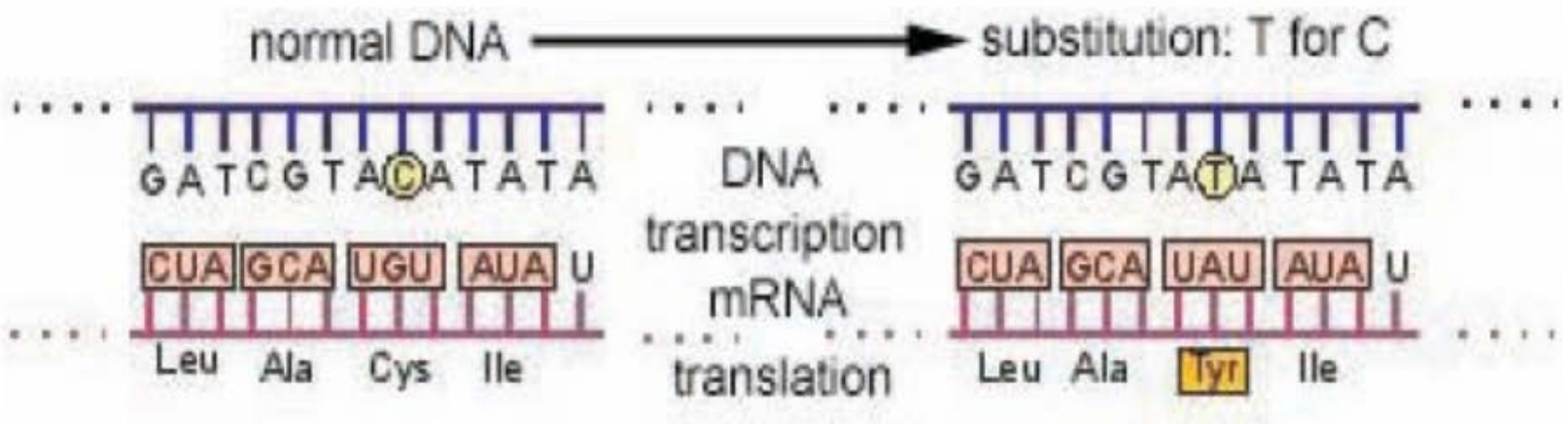
i) Point Mutation or Substitution of a Nucleotide



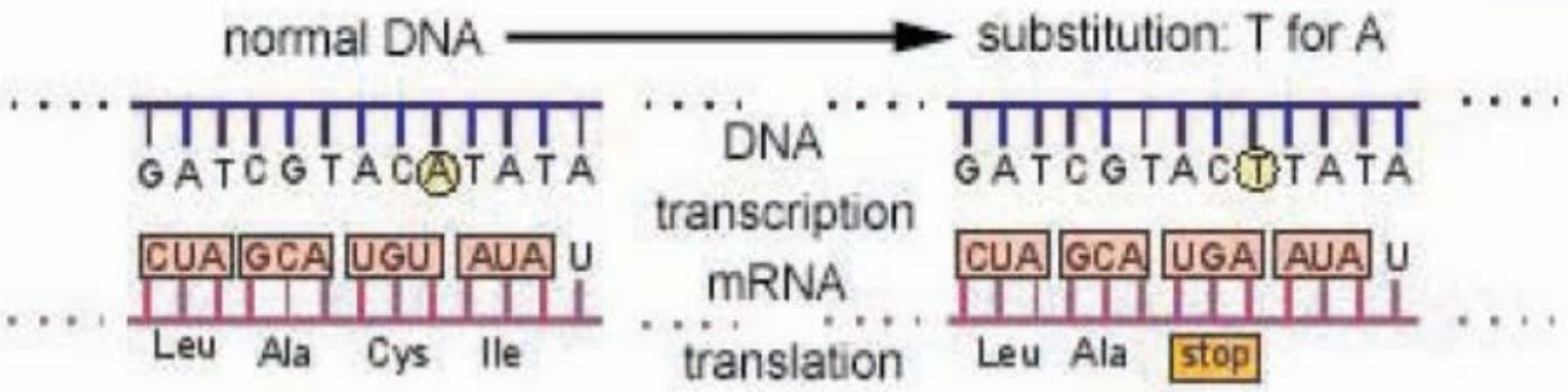
ii) Deletion of a nucleotide



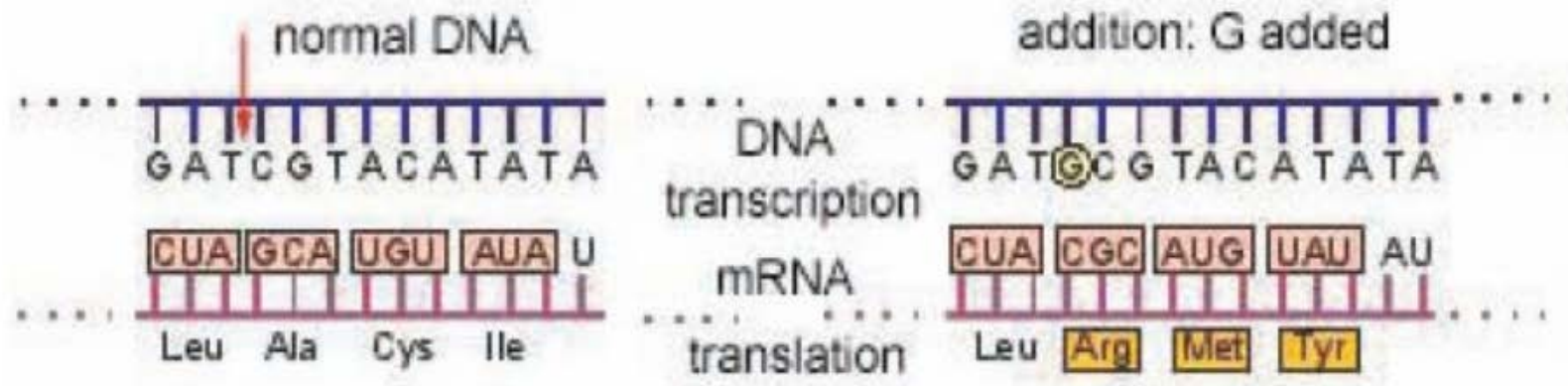
iii) Addition of a Nucleotide



iv) Substitution of a nucleotide: Results in one wrong codon and one wrong amino acid



v) Substitution of a nucleotide: Results in a 'stop' codon and premature termination of the protein



vi) Frameshift mutation: Results in a reading frame shift. All codons.

The practical isolation of mutants

(i) ***Exposing organisms to the mutagen*:**

- The organism undergoing mutation should be in the haploid stage during the exposure.
- Bacterial cells are haploid; in fungi and actinomycetes the haploid stage is found in the spores.

(ii) ***Selection for mutants*:**

- The selection of mutants is greatly facilitated by relying on the morphology of the mutants or on some selectivity in the medium.

- When morphological mutants are selected, it is in the hope that the desired mutation is pleotropic (i.e., a mutation in which change in one property is linked with a mutation in another character).
- The classic example of a pleotropic mutation is to be seen in the development of penicillin-yielding strains of *Penicillium chrysogenum*.
- It was found in the early days of the development work on penicillin production that after irradiation, strains of *Penicillium chrysogenum* with smaller colonies and which also sporulated poorly were better producers of penicillin.
- Similar increases of metabolite production associated with a morphological change have been observed in organisms producing other antibiotics: cycloheximide, nystatin, and tetracyclines.

- In-built selectivity of the medium for mutants over the parent cells may be achieved by manipulating the medium.

- If, for example, it is desired to select for mutants able to stand a higher concentration of alcohol, an antibiotic, or some other chemical substance, then the desired level of the material is added to the medium on which the organisms are plated.

- Only mutants able to survive the higher concentration will develop.

- For example, we need special bacteria to degrade specific pollutant substance.

- To find the most efficient one among them, we can grow them on selective media, which contain increasing concentrations of pollutant.

- However, as the concentration increase, the number of surviving bacteria will decrease.

- The concentration of the toxic pollutant could be gradually increased in the growth medium thus selecting the most resistant ones. This method is called acclimatization.

Isolation of auxotrophic mutants

- **Auxotrophic mutants** are those which lack the enzymes to manufacture certain required nutrients; consequently, such nutrients must therefore be added to the growth medium.
- In contrast the wild-type or prototrophic organisms possess all the enzymes needed to synthesize all growth requirements.
- As auxotrophic mutants are often used in industrial microbiology, e.g., for the production of amino acids, nucleotides, etc.

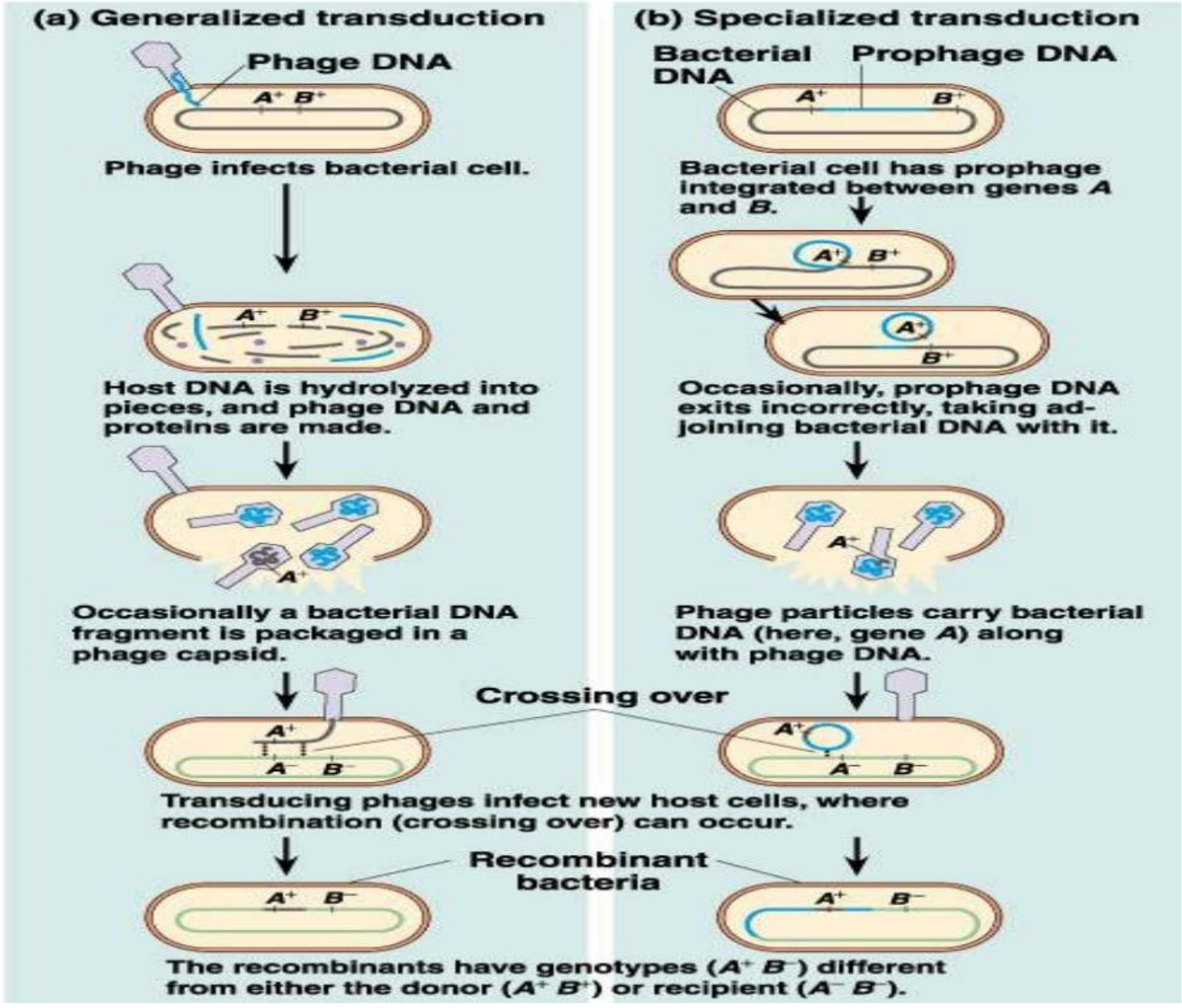
Reports on strain improvement by mutation-

- Karana and Medicherla (2006)- lipase from *Aspergillus japonicus* MTCC 1975- mutation using UV, HNO_2, NTG showed 127%, 177%, 276% higher lipase yield than parent strain respectively.
- Sandana Mala *et al*., 2001- lipase from *A. niger* - Nitrous acid induced mutation – showed 2.53 times higher activity.

- Medically useful products Demethyltetracycline and doxorubicin were discovered by mutations from tetracycline and daunorubicin(Shir et al,1969).Hybramycines were also made by this way.
- First superior penicillin producing mutant,*Penicillium chrysogenum* X-1612,was isolated after X ray

Transduction

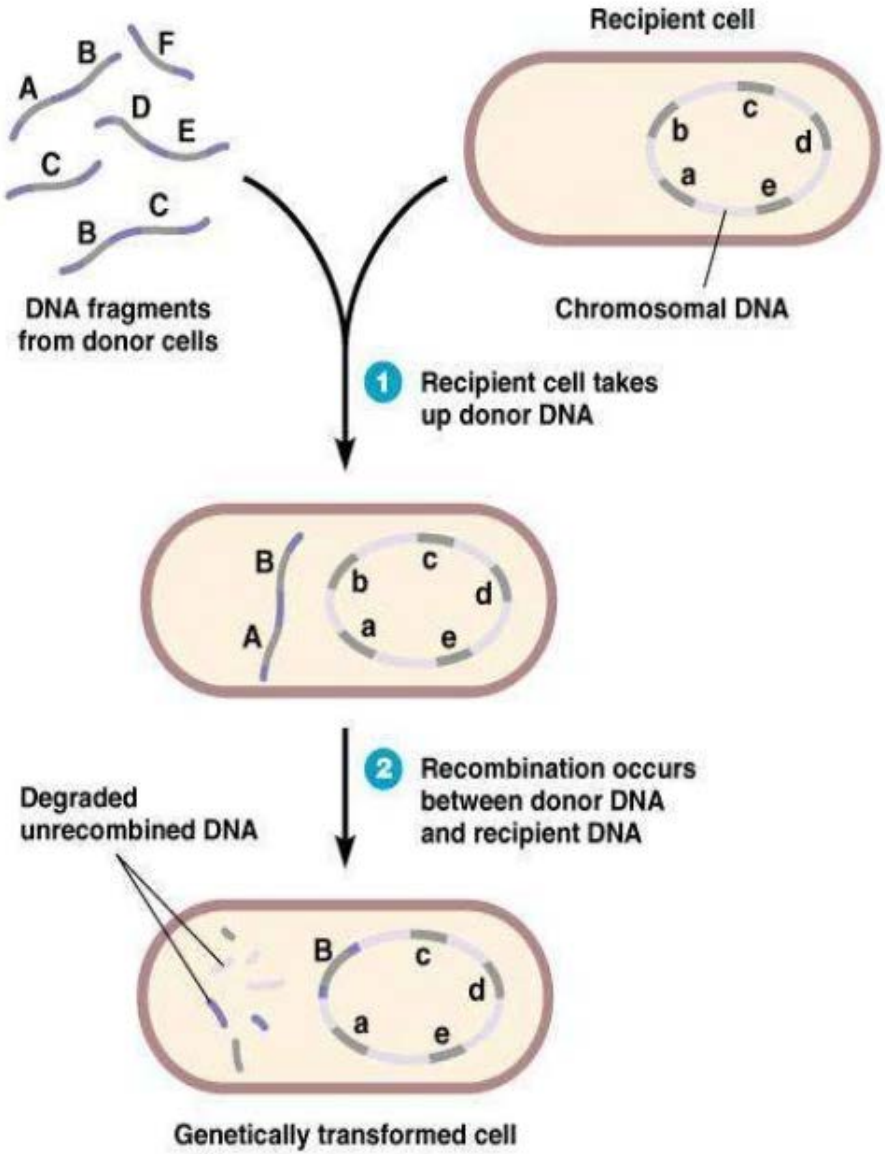
- Transduction is the transfer of bacterial DNA from one bacterial cell to another by means of a bacteriophage.
- Two types:
  - *general transduction* and
  - *specialized transduction*.
- In general transduction, host DNA from any part of the host's genetic apparatus is integrated into the virus DNA.
- In specialized transduction, which occurs only in some temperate phages, DNA from a specific region of the host DNA is integrated into the viral DNA and replaces some of the virus' genes.
- The method is a well-established research tool in bacteria including actinomycetes but prospects for its use in fungi appear limited.


(a) Generalized transduction
Phage DNA
A+ B+
Phage infects bacterial cell.
A+ B+
Host DNA is hydrolyzed into pieces, and phage DNA and proteins are made.
A+
Occasionally a bacterial DNA fragment is packaged in a phage capsid.
(b) Specialized transduction
Bacterial DNA
Prophage DNA
A+
B+
Bacterial cell has prophage integrated between genes A and B.
A+ B+
A+
B+
Occasionally, prophage DNA exits incorrectly, taking adjoining bacterial DNA with it.
A+
Phage particles carry bacterial DNA (here, gene A) along with phage DNA.
Crossing over
A+
A− B−
A+
A− B−
Transducing phages infect new host cells, where recombination (crossing over) can occur.
Recombinant bacteria
A+ B−
A+ B−
The recombinants have genotypes (A+ B−) different from either the donor (A+ B+) or recipient (A− B−).

Transformation

- **Bacterial transformation** is a process of horizontal gene transfer by which some **bacteria** take up foreign genetic material (naked DNA) from the environment.

- Cells in which transformation can occur are 'competent' cells.



- In some cases competence is artificially induced by treatment with a calcium salt.

- The method has also been used to increase the level of protease and amylase production in *Bacillus* spp.

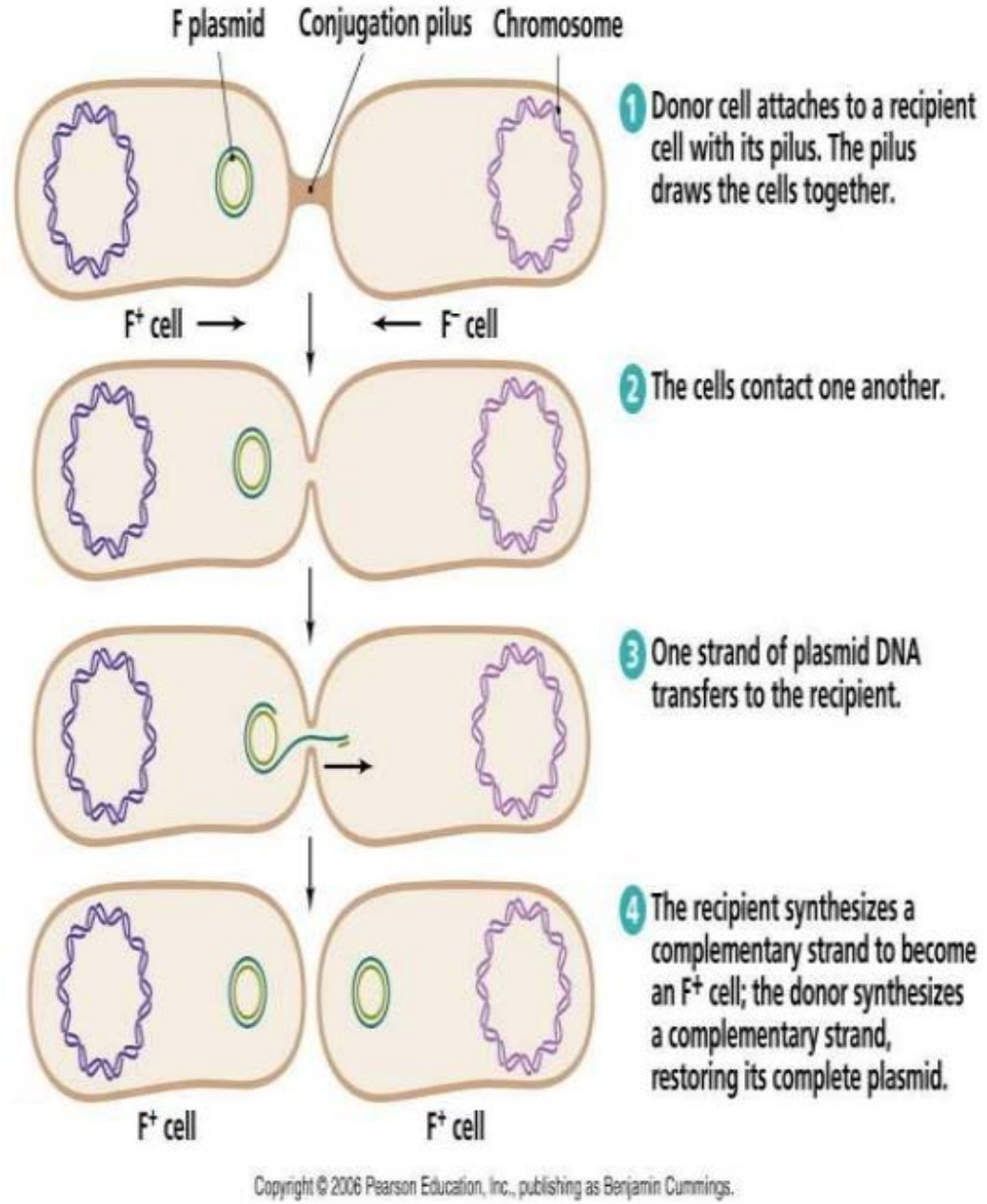
- The method therefore has good industrial potential.

Conjugation

Bacterial conjugation : is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells.

Conjugation types:

1) F^+ x F^- Conjugation
2) Hfr x F^- Conjugation
3) F’ x F^- conjugation



Protoplast fusion

- Protoplasts are formed from bacteria, fungi, yeasts and actinomycetes when dividing cells are caused to lose their cell walls.
- Fusion from mixed populations of protoplasts is greatly enhanced by the use of polyethylene glycol (PEG).
- The method has great industrial potential and experimentally has been used to achieve higher yields of antibiotics through fusion with protoplasts from different fungi.
- Protoplast fusion has been demonstrated as an efficient way to induce hetero-karyon formation and recombination with high frequency

Reports on strain improvement by protoplast fusion

- Kim *et al*., 1998 did a comparative study on strain improvement of ***Aspergillus oryzae*** for protease production by both mutation and protoplast fusion.

--UV radiation – 14 times higher yield.

--Ethyl methane sulphonate – 39 times higher yield.

--Protoplast fusion – using PEG and CaCl2 – 82 times higher yield.

- An intergeneric hybrid was obtained from ***Aspergillus niger*** and ***Penicillium digitatum*** for enhancing the production of verbenol,a highly valued food flavorant (Rao *et a*l, 2003)

Parasexual recombination

- Parasexuality is a rare form of sexual reproduction which occurs in some fungi.
- In parasexual recombination of nuclei in hyphae from different strains fuse, resulting in theformation of new genes.
- Parasexuality is important in those fungi such as *Penicillium chrysogenum* and *Aspergiluss niger* in which no sexual cycles have been observed.

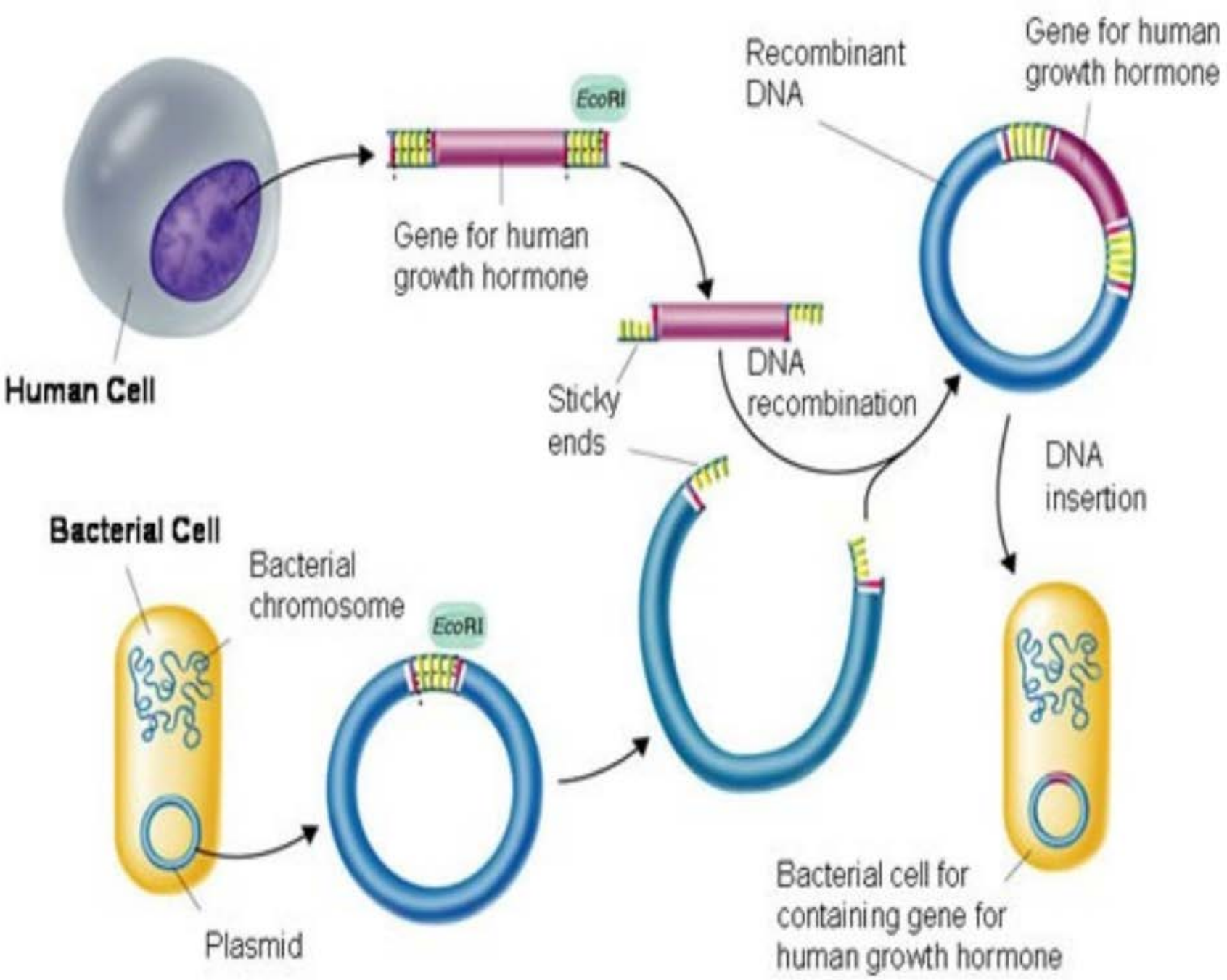
- It has been used to select organisms with higher yields of various industrial product such as phenoxy methyl penicillin, citric acid, and gluconic acid.

- Parasexuality has not become widely successful in industry because the diploid strains are unstable and tend to revert to their lower-yielding wild-type parents.

- More importantly is that the diploids are not always as high yielding as the parents.

Genetic Engineering

- **Genetic engineering (GE)** is the process of using recombinant DNA (rDNA) technology to alter the **genetic** makeup of an organism.
- **Genetic engineering** is accomplished in three basic steps:
  - The isolation of DNA fragments from a donor organism
  - The insertion of an isolated donor DNA fragment into a vector genome
  - The growth of a recombinant vector in an appropriate host.


Human Cell
EcoRI
Gene for human growth hormone
Sticky ends
DNA recombination
Recombinant DNA
Gene for human growth hormone
DNA insertion
Bacterial Cell
Bacterial chromosome
EcoRI
Plasmid
Bacterial cell for containing gene for human growth hormone

Improvement of microbial processes by GE

Primary metabolites

- New processes for the production of amino acids and vitamins have been developed by recombinant DNA technology.
- *Escherichia coli* strains were constructed with plasmids bearing amino acid biosynthetic operons.
- Cloning extra copies of threonine export genes into *E. coli* led to increased **threonine** production.
- An engineered strain of *Corynebacterium glutamicum* producing **L -tryptophan** was further modified by cloning in additional copies of its own transketolase gene.
- **Biotin** has been made traditionally by chemical synthesis but recombinant microbes have approached a competitive economic position. The cloning of a biotin operon (*bioABFCD)* on a multicopy plasmid allowed *E. coli* to produce 10000 times more biotin than did the wild-type strain.
- **Riboflavin** production in *Corynebacterium moniagenes* - was developed by cloning and overexpressing the organism's own riboflavin biosynthesis genes and its own promoter sequences.
- A novel process for **vitamin C** synthesis involved the use of a genetically engineered *Erwinia herbicola* strain containing a gene from *Corynebacterium* sp.

Improvement of microbial processes by GE

- **Secondary metabolites**
- Studies revealed that many antibiotic biosynthesis genes were arranged in clusters.
- The entire cephamycin C pathway was cloned and expressed from a cephamycin-producing strain of *Streptomyces cattleya* into another cephamycin producer, *Streptomyces lactamgens* , a two- to three-fold improvement was obtained.
- **Microbial enzymes**
- Genes encoding many microbial enzymes have been cloned and the enzymes expressed at levels hundreds of times higher than those naturally produced.
- Scientists at Novo Nordisk isolated a very desirable lipase for use in detergents from a species of *Humicola*.
- For production purposes, the gene was cloned into *Aspergillus oryzae* , where it produced 1000-fold more enzyme and is now a commercial product.
- The α-amylase gene from *Bacillus amyloliquefaciens* was cloned using multicopy plasmid pUB110 in *B. subtilis*

Improvement of microbial processes by GE

Polymers, fuels, foods and beverages

- Recombinant DNA manipulation of *Xanthomonas campestris* increased titers of xanthan by two-fold.
- Alcohol dehydrogenase II and pyruvate decarboxylase genes from *Zymomonas mobilis* were inserted in *E. coli.*
- Beer wort contains barley β-glucans which reduce the filtrability of beer and lead to precipitates and haze in the final product. The gene coding for endoglucanase was transferred from *Trichoderma reesei* to brewer's yeast and the engineered yeast strain efficiently hydrolyzed the β-glucans.

Bioconversions

- Recombinant *Candida pasteurianum* can carry out the conversion of glycerol to 1,3-propanediol.

Questions

- Write short note no novel genetic technologies for strain improvement of industrially important microorganisms.
- What is strain improvement in industrial microbiology?
- Write as assay on strategies of strain improvement of industrial microorganisms.
- Write a short note on mutation for strain improvement.
- Recombinant DNA technology as tool for strain improvement of industrial microorganisms.
- Explain recombination method for strain improvement