# Strain Development


**References**

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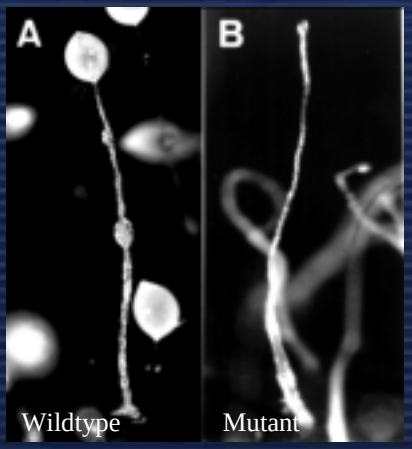
## To develop the procedures for obtaining new microbial metabolites

- Screening
  - Selection for the production of new metabolites with new isolates and/or new test methods
- Chemical modification
  - Modification of known microbial substances
- Biotransformation
  - Change in a chemical molecule by means of a microbial or enzyme reaction
- Interspecific protoplast fusion
  - Recombination of genetic information from rather closely related producer strains. New or hybrid substances are expected
- Gene cloning
  - Genes may be transferred between unrelated strains which are producers of known substances

## Objective of a genetic strain development

- The objective of a genetic strain development from wild strains to mutants depends on the process

Bio industrial Process

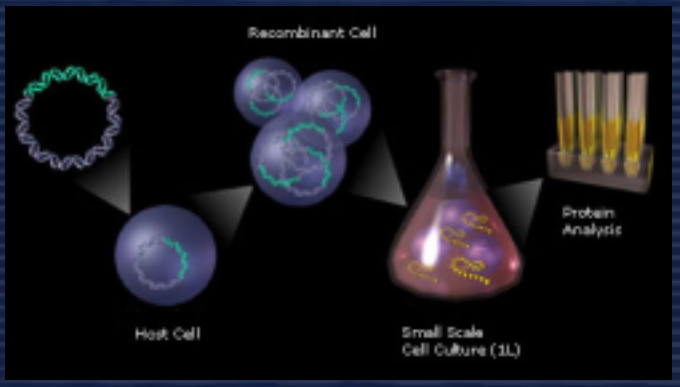



## Industrial strain development

- The major motivation for industrial strain development is economic, since the metabolite concentrations produced by wild strains are too low for economical processes
- Depending on the system, it may be desirable to isolate strains which require shorter fermentation times, which do not produce undesirable pigments, which have reduced oxygen needs, which exhibit decreased foaming during fermentation, or which are able to metabolize inexpensive substrates.
- Wild strains frequently produce a mixture of chemically closely related substances. Mutants which synthesize one component as the main product are preferable, since they make possible a simplified process for product recovery.
- Changes in the genotype of microorganisms can lead to the biosynthesis of new metabolites. Thus, mutants which synthesize modified antibiotics may be selected

## Recombinant DNA techniques

- One of the most significant approaches to strain Improvement
- Recombinant DNA techniques - Bringing together in one organism genes from several organisms has the potential for not only increasing yields but also for producing entirely new substances.




## Mutation

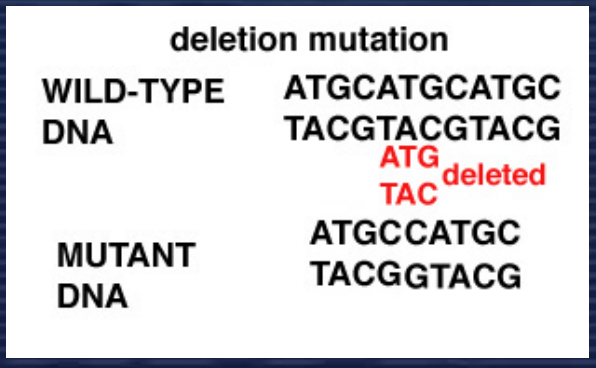
- Changes in the genotype are caused by mutation and genetic recombination.
- The success of strain development depends on an optimal use of mutagenesis (production of mutations) procedures in combination with an effective system for selecting high-yielding strains.
- Genotype: The genetic makeup of an organism.
- Phenotype: The physical appearance and the observable properties of an organism that are produced by the interaction of the genotype with the environment.

## Spontaneous and induced mutations

- Mutation occurs spontaneously or after induction with mutagenic agents (mutagens). The rate of spontaneous mutation depends on the growth conditions of the organism and is between 10-10 and 10-6. The mutation frequency (proportion of mutants in the population) can be significantly increased by using mutagenic agents up to 10-5 - 10-1
  - Genome Mutation : May cause changes in the number of chromosomes
  - Chromosome mutation : May change the order of the genes within the chromosome (deficiency, deletion, inversion, duplication, traslocation)
  - Gene or point mutations : May result from changes in the base sequence in a gene

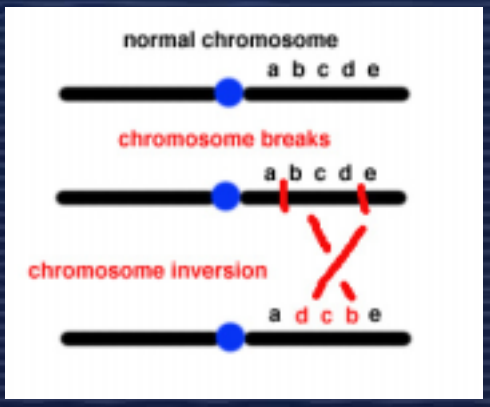
## Chromosome Mutation

- Deletion: A point mutation in either RNA or DNA in which a single nucleotide is removed from a polynucleotide strand



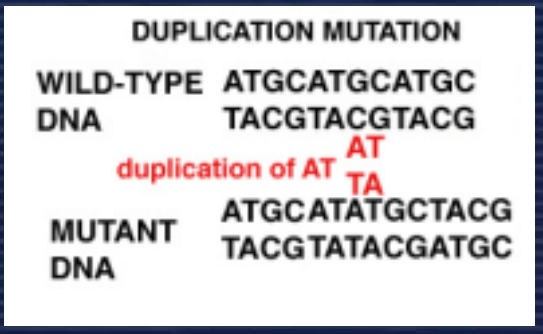
## Inversion Mutation

Inversion: A chromosomal aberration in which a block of genes is rotated by 180° so that the sequence of genes in that block is inverted



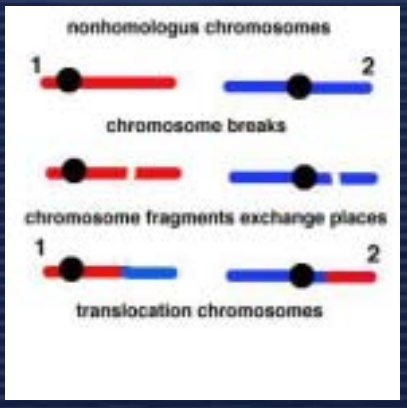

## Duplication Mutation

- Duplication: A chromosomal aberration in which a chromosome bears two identical groups, each composed of one or several genes.



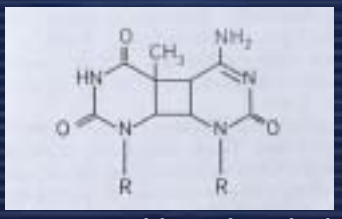
## Traslocation Mutation

- Translocation: An interchromosomal aberration in which a chromosome fragment becomes inserted into another




## Mutagenesis through radiation

- **Wave length**: 200-300 nm, with an optimum at 254 nm, which is the absorption maximum of DNA. The most important products of UV action are dimers(thymine-thymine, thymine-cytosine and cytosine-cytosine) formed between adjacent pyrimidines or between pyrimidines of complementary strands, which results in crosslinks. UV mainly induces trasitions of GC --> AT; transversions, frameshift mutations and deletions are also found.

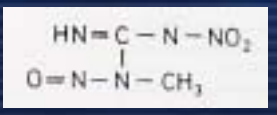



Thymine-cytosine-cyclobutane dimer, the photoproduct
Formed as a result of UV radiation

- **transition**: An exchange of a purine with another purine or a pyrimidine with another pyrimidine
- **transversion**: The substitution of a pyrimidine with a purine or vice versa
- **frameshift mutations**: When one nucleotide or more is inserted or deleted, thus altering the reading frame in the following transcription and translation processes, and leading to a changed amino acid sequence in the resulting protein.

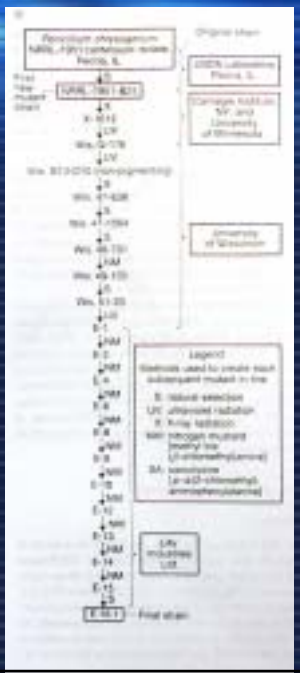
## Mutagenesis with chemical agents

- NTG(N-methyl-N'-nitro-N-nitrosoguanidine); One of the most effective chemical mutagens. 90% of the mutations induced by NTG are GC -->AT transitions; to a small extent deletions and frameshift mutations



structure of NTG

## NTG Mutation



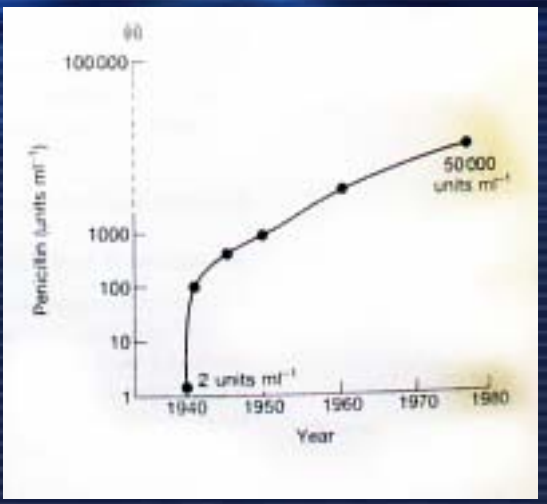
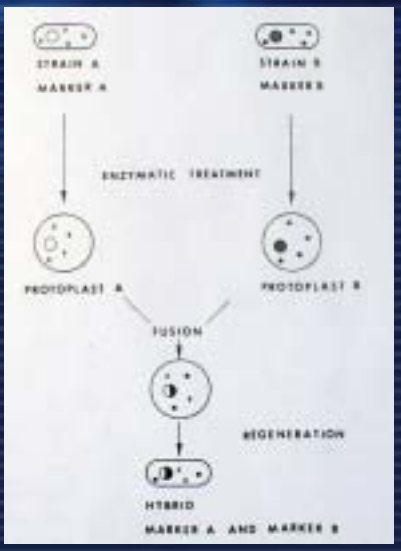



Fig. left) Development of a high penicillin producing Strain via genetic manipulation.
Right) Evolution of the penicillin fermentation

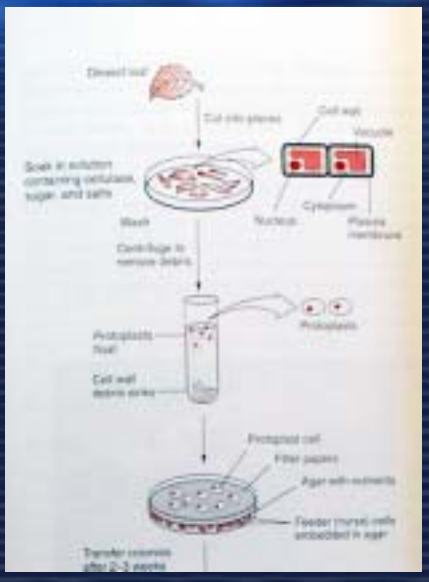
# Protoplast fusion

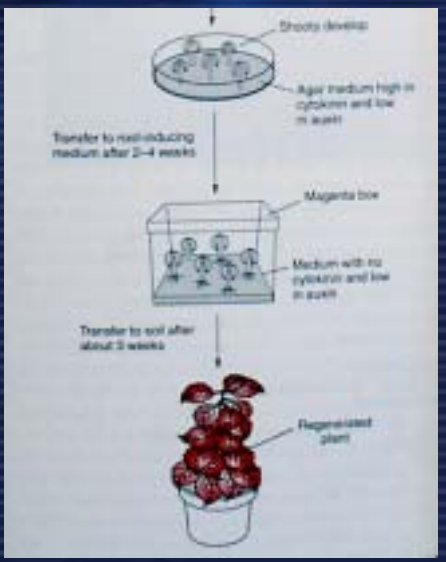
- Fusion(Hybridization): The fusion of two cells in tissue culture.
  - Cell wall is removed by an enzymatic treatment(protoplast)
  - The two different strains after removal of cell wall forced to fuse using polyethylene glycol(PEG).
  - After the fusion, the cells are allowed to regenerate their cell wall.



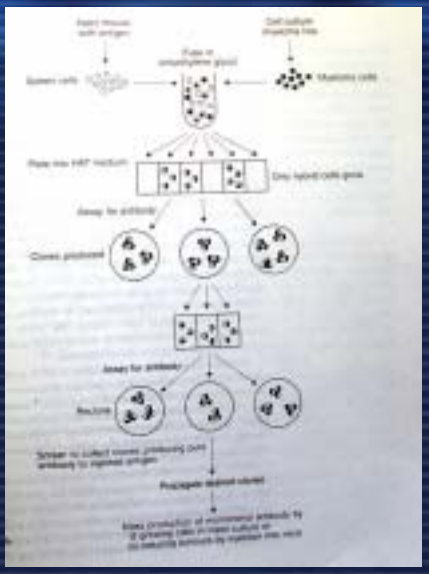

protoplast fusion technique for hybrid yeast

# Protoplast fusion and regeneration of a hybrid plant

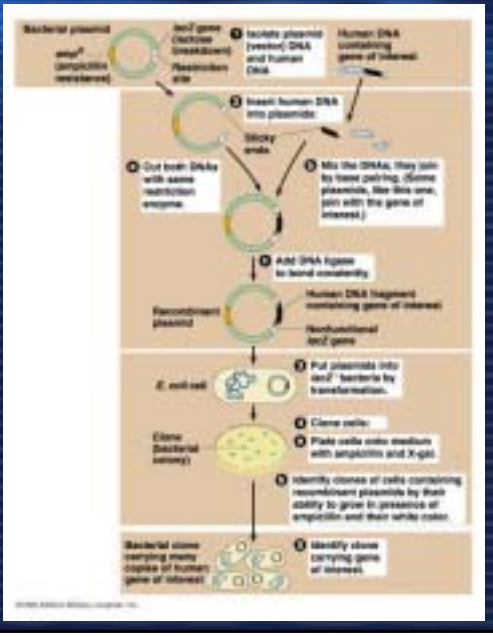




## Mammalian cell fusion

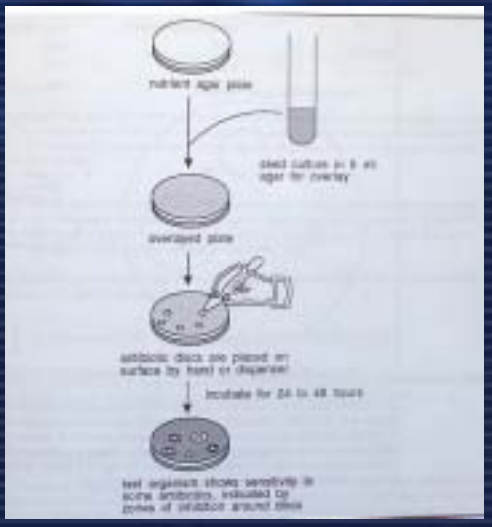


## Gene Cloning



## Selection of Mutants

- Random screening
  - Repeated mutation and selection. A gradual increase in the yield is attained by continuing with these steps.
  - After a series of mutation, the accuracy of the screening test is required against pathogens or toxic substances
- MIC(Minimum Inhibitory Concentration) : The minimum a\mount needed to inhibit growth of a test organism

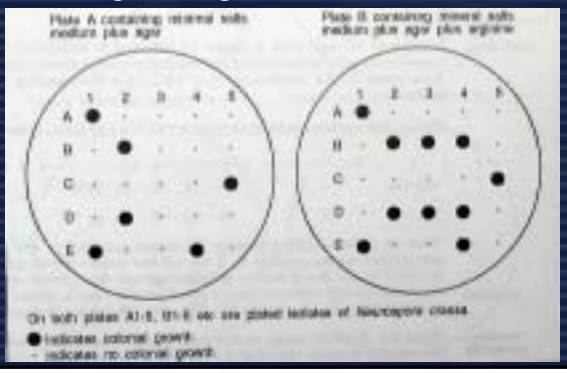


## Selective isolation of mutants

- Auxotrophic mutant (See Genetic Engineering Section)
  Enrichment
  - For bacteria, cultivation in minimal medium with the addition of an antibiotic which acts only on growing cells(e.g., penicillin).
  - For fungi, cultivation in minimal medium and filtration off of the germinated mycelia.
- Recognition test
  - For bacteria, plating out on complete medium and replica plating on to minimal medium with various supplements.
  - For fungi, plating out on complete medium and replating on minimal medium with the filter paper replica technique. Testing of suspects on minimal medium with various supplements.

## Selective isolation of mutants

- Auxotrophic mutant: The mutants of particular interest were those which had lost the ability to produce just a single enzyme. It was found that some of these mutants would grow if the medium was supplemented with a specific metabolite, the biosynthesis of which had been impaired.
  - Replica plating: This technique could involve plating up to 25 isolates into the usual 10 cm plate containing minimal salts medium plus agar. These isolates are plated in the same order onto a second plate containing minimal medium, agar and arginine.




## Resistant Mutant

- See genetic engineering section enrichment
  - Plating out a large number of cells on solid nutrient media with the addition of
  - the inhibiting substance.
  - Recognition test)
  - Direct, since only the resistant cells grow in colonies.

# Temperature sensitive mutant

- At high temperatures, plating out cells on solid nutrient media.
- Recognition test
- Direct, since only temperature-sensitive mutants grow.