



Strain improvement

MICROBIAL CONCEPTS

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In industries the micro-organism are selected by using various screening procedure. (Primary/secondary screening)

For obtaining **constant high yield** of product the industries carry out strain improvement as well as strain selection programs continuously.
During the strain improvement program various parameters are adjusted to increase product yield.

The strain improvement of micro-organism used in fermentation process is done by altering the genetic make up of strain. Other than working on DNA, parameter and media optimization are also used.

Following methods are used for Strain improvement:

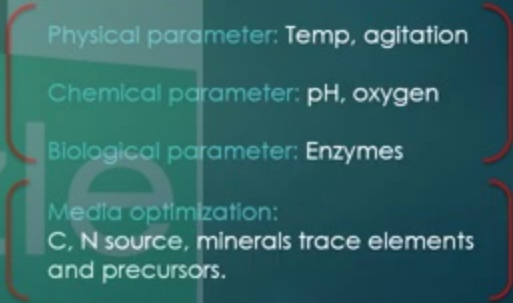
- Genetic recombination or gen transfer.
- Mutation
- Genetic engineering

Physical parameter: Temp, agitation

Chemical parameter: pH, oxygen

Biological parameter: Enzymes

Media optimization:
C, N source, minerals trace elements and precursors.



Mutation

Mutation can be defined as change in genetic structure of micro-organism.

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The changing of the structure of a gene, resulting in a variant form that may be transmitted to subsequent generations, caused by the alteration of single base units in DNA, or the deletion, insertion, or rearrangement of larger sections of genes or chromosomes.

The ability of micro-organism to produce a desired product can be enhanced by a mutation process. In mutation process the most stable and efficient strains are exposed to **mutagenesis** (process) by using different **mutagenic agents** (mutagens).

The mutagenic agents like **ionization, ultraviolet radiation, acids and alkalis** are used in mutation.

Result of mutation obtain should be - **increased in yield of desired product** and decreased yield of undesired product.

The mutagenic agents are used in such concentration that maximum number of cells die due to mutagenic agents and only the that are capable to carry out mutation and have the capacity to tolerate the levels of mutagenic agents are able to survive.

From the survived cells the cells which are undergone mutation and have the capacity to produce high yield of fermented product is selected.

Relevant strategies for selection of mutants:

isolation of auxotrophic mutants:

amino acid production from the bacterium *Corynebacterium glutamicum*.

An auxotrophic mutant has a defect in one of its biosynthetic pathways so that it requires a specific biochemical for normal growth and development.

For example, phe^- mutants require phenylalanine for growth; such mutants of *C. glutamicum* accumulate tyrosine. Similarly, tyr^- mutants accumulate phenylalanine, while $\text{phe}^- + \text{tyr}^-$ mutants accumulate tryptophan.

(ii) Many analogue-resistant mutants have feed-back insensitive enzymes of the biosynthetic pathway the analogue of whose product was used for selection of such cells. In feed-back inhibition, activity of an enzyme is inhibited by the end-product of the biosynthetic pathway in which the enzyme participates.

For example, when tyr^- mutants of *C. glutamicum* were selected for resistance to 50 mg/l p- fluorophenylalanine (analogue of phenylalanine), there was a nearly 7-fold increase in phenylalanine accumulation over that of the tyr^- mutant.

(iii) Sometimes revertants from nonproducing mutants of a strain are high producers, e.g., one such reversion mutant of *Streptomyces viridifaciens* showed over 6-fold increase in chlortetracycline production over the original strain from which the nonproducing mutant was obtained. When a mutant mutates back to its original phenotype it is called reversion, and the mutant is known as revertant, e.g., non-producer mutant mutating back to producer phenotype.

Genetic engineering technique is the most successful technique used for strain improvement programs on industrial scale.

It is also called as Recombinant DNA technology and in this process there is alteration of genetic characters of cells and hence result in change in phenotypic character of cell.

In this technique a desired type of gene is introduced from one microbial cell to other microbial cells by using cloning vectors, like plasmids, phages.

A specific and desired character of a microbial cell can be introduced in the selected strain to improve the yield of product.

This technique has been used to achieve the following two broad objectives:

- (i) production of recombinant proteins, and
- (ii) modification of the organism's metabolic pattern for the production of new, modified or more quantity of metabolites (metabolic engineering).



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