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Strain Improvement and Preservation

Introduction

❖**Strain-** A Strain is a group of species with one/ more characteristics that distinguish it from other sub groups of the same species of the strain .
- each strain is indentified by a name, number or letter.
Example:- E.coli Strain K12

The Science and technology of manipulating and improving microbial strains, in order to enhance their metabolic capacities for biotechnological applications, are referred to as strain improvement.

❖**Strain Improvement-**The Science and Technology of manipulating and improving microbial strains in order to enhance their metabolic capacities is known as Strain Improvement

Ideal Characteristics of Strain

- Rapid growth
- Genetic stability
- Non-toxicity to humans
- Ability to use cheaper substrates
- Elimination of the production of compounds that may interfere with downstream processing
- To improve the use of carbon and nitrogen sources.
- Reduction of cultivation cost
- Shorter fermentation time.

Purpose of Strain Improvement

- Increase the productivities
- Regulating the activity of the enzymes
- Increasing the permeability
- To change un used co-Metabolites
- Introducing new genetic properties into the organism by Recombinant DNA technology / Genetic engineering.

Methods of Strain Improvement

- Mutant Selection
- Recombination
- Recombinant DNA Technology

MUTANT SELECTION

- A MUTATION is a Sudden and Heritable change in the traits of an organism.
- Application of Mutagens to Induce mutation is called MUTAGENESIS.
- Agents capable of inducing mutations are called MUTGENS Physical – Particulate and Non-Particulate

Chemical – Base analog, Deamine & Alkylating agents, Acridine Dyes.

- Mutation occurring without any specific treatment are called "Spontaneous Mutation."

- Mutation are resulting due to a treatment with certain agents are known as "Induced Mutation."

- Many Mutations bring about marked changes in the Biochemical Characters of practical interest these are called Major Mutations – these can be used in Strain Improvement

Ex: *Streptomyces griseus*-Streptomycin-Mannosidostreptomycin Ex: *Streptomyces aurofaciens*(S-604) –
Produce 6-demethyl tetracycline in place of Tetracycline

- In contrast, most improvements in biochemical production have been due to the Stepwise accumulation of so called Minor genes.

Ex: *Pencillium chrysogenum* – Strain E15-1 was obtained which yield 55% more penicillin than original strain

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

Isolation of Mutant

Isolation of Auxotrophic Mutants:- it has a defect in one of its biosynthetic pathways, so it require a specific Bio-molecule for normal growth & development.

Ex: Phe^- mutant of C.glutamicus – require Phe for growth so, it accumulates Tyrosine.

2 **Analogue – Resistant Mutant:-** it have feed back insensitive enzymes of the biosynthetic pathway.

Feed - back inhibition- Tyr^- mutant of C. glutamicus were selected for resistance to 50mg/L of p-flurophenylalanine (analogue of phenylalanine).

3 **Revertants from non producing mutants**:- Of a Strain are high producer . Mutant mutates back to its original phenotype is called Reversion and mutant is called Revertant.

Ex: Reversion mutant of *Streptomyces viridifaciens* showed over 6-fold increase in chlortetracycline production over the original strain.

RECOMBINATION

- Defined as formation of new gene combinations among those present in different strains.
- Recombination used for both genetic analysis as well as strain improvement
- To generate new products
- Recombination may be based on:-
  - Cross over
  - Transformation
  - Conjugation
  - Transduction
  - protoplast fusion – The fusion between non producing strains of two species (*Streptomyces griseus* and *Streptomyces tenjimariensis*) has yielded a strain that produces indolizomycin, a new Indolizine antibiotic.

RECOMBINATION DNA TECHNOLOGY

rDNA Technology or Genetic Engineering involves the isolation and cloning of genes of interest, production of the necessary gene constructs using appropriate enzymes and then transfer and expression of these genes into an suitable host organism.

This technique has been used to achieve 2 broad objectives:

- Production of Recombinant proteins
- Metabolic Engineering

1. Recombinant proteins:- These are the proteins produced by the transferred gene / transgene ; they themselves are of commercial value.
Ex: Insulin, Interferons etc..are produced in Bacteria

2. Metabolic Engineering :- When metabolic activities of an organism are modified by introducing into it transgenes, which affect enzymatic, transport and /or regulatory function of its cells its known as Metabolic Engineering
Examples – Over production of the amino acid Isoluecine in C. glutamicum& Ethanol by E.coli

Strain Preservation

- Industrial Microbiology continuously uses specific Microorganisms isolates/strains as research, assay, development and production cultures.

- These strains are highly valuable and must be preserved over long periods without genetic and phenotypic changes.

  - Research culture
  - Assay culture
  - Development culture
  - Production culture

Approaches of Strain Preservation

- **Low Temperature Storage**:- 2-6°c (2-6 months)
- **Storage as Frozen Culture**:- -20 to -100°c.
- **Storage as Lyophilized cells**:- Under high Vacuum at low temperature (5/ even -20 to -70°c)
- **Storage of Vegetative cells/spores in Liquid Nitrogen:-** - 196°c / -167°c .
- **Air dried at room temperature** on sterile loam sand or on other natural substrate:-Like maize seed, rice, bran, etc.,

(bacterial culture may remain viable up to 70-80 years)

- **Storage in Glycerin Stabs:-** 0.85 ml of cell suspension mixed with0.15ml of sterile glycerol and stored at- 70 or -75°c.