RAMA UNIVERSITY

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FACULTY OF ENGINEERING & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

Isolation and Screening of Industrial Strain

•Isolation of from the environment is by:

•Collecting samples of free living microorganism from anthropogenic or natural habitats.

•These isolates are then screened for desirable traits.

•Or by sampling from specific sites:

•Microorganism with desired characteristics are found among the natural microflora

•After sampling of the organism the next step is of enrichment.

Enrichment:

Enrichment in batch or continuous system on a defined growth media and cultivation conditions are performed to encourage the growth of the organism with desired trait. This will increase the quantity of the desired organism prior to isolation and screening.

Screening

•Subsequent isolation as pure cultures on solid growth media involves choosing or developing the appropriate selective media and growth conditions.

•Next step to enrichment and isolation is Screening.

•The pure cultures must be screened for the desired property; production of a specific enzyme, inhibitory compound, etc.

•Selected isolates must also be screened for other important features, such as stability and, where necessary, non-toxicity.

•These isolation and screening procedures are more easily, applied to the search for a single microorganism.

The industrial microorganism should ideally exhibit:

1. genetic stability

2. efficient production of the target product, whose, route of biosynthesis, should preferably be well characterized.

3. limited or no need for vitamins and additional growth factors.

4. utilization of a wide range of low-cost and readily available carbon sources

5. amenability to genetic manipulation;

6. safety, non-pathogenic and should not produce toxic agents, unless there is the target product;

7. ready harvesting from the fermentation; .

8. production of limited byproducts to ease subsequent purification problems.

Culture Preservation

▪Streptomyces aureofaciens NRRL 2209 was the first microorganism deposited in a culture collection in support of a microbially based patent application.

▪Preservation of microbial cultures was critical for all individuals and firms engaged in the search for patentable products from and patentable processes by microorganisms

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- Preservation of cultures by freezing, drying, or a combination of the two processes is highly influenced by resistance of the culture to the damage caused by rapid freezing, the dehydrating effects of slow freezing, or damage caused during recovery.

- To minimize damage, agents have been used that protect against ice formation by causing the formation of glasses upon cooling.

▪Methods to protect against the negative effects of dehydration include adaptation to lower effective water activity by pre-incubation in high osmotic pressure solutions.

▪Damage caused by thawing after freezing can be minimized by rapid melting and by the composition of the medium used for growth after preservation.

There are various preservation methods .

To date, preservation in liquid nitrogen is still the most successful long-term method.

Serial Transfer

•Based upon its ease of use, serial transfer is often the first “preservation” technique used by microbiologists.

•The disadvantages of relying upon this method for culture maintenance include contamination, loss of genetic and phenotypic characteristics, high labor costs, and loss of productivity.

Preservation in Distilled Water

•This method (Castellani method, 50 years ago) was extensively tested on 594 fungal strains:

•62% of the strains growing and maintaining their original morphology.

•In another study, 76% of yeasts, filamentous fungi, and actinomycetes survived storage in distilled water for 10 years.

Preservation under Oil

- One of the earlier preservation methods was the use of mineral oil to prolong the utility of stock cultures.
- Mineral oil has been found to prevent evaporation from the culture and
- Decrease the metabolic rate of the culture by limiting the supply of oxygen.
- This method is more suitable than lyophilization for the preservation of non-sporulating strains.

Lyophilization

•One of the best methods for long-term culture preservation of many microorganisms is freeze-drying (lyophilization).

•The commonly used cryoprotective agents are skim milk (15% [wt/vol] for cultures grown on agar slants and 20% for pelleted broth cultures) or sucrose (12% [wt/vol] final concentration).

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•It should be noted that some plasmid--containing bacteria are successfully preserved by this method.

- **Storage over Silica Gel**

- Neurospora has successfully been preserved over silica gel.

- **Preservation on Paper**

- Drying the spores on some inert substrates can preserve spore-forming fungi, actinomycetes, and unicellular bacteria.

- Fruiting bodies of the myxobacteria, containing myxospores, may be preserved on pieces of sterile filter paper and stored at room temperature or at 6°C for 5 to 15 years.

- **Preservation on Beads**

- The method involving preservation on beads (glass, porcelain) , developed by Lederberg, is successful for many bacteria.

Liquid Drying

- To avoid the damage that freezing can cause, a liquid—drying preservation process is applied.
- It has effectively preserved organisms such as anaerobes that are damaged by or fail to survive freezing.
- This procedure was preferred over lyophilization for the maintenance of the biodegradation capacity of six gram--negative bacteria capable of degrading toluene.
- Malik's liquid-drying method was also found to be markedly superior to lyophilization for the preservation of unicellular algae.

Cryopreservation

•Microorganisms may be preserved at - 5 to - 20°C for 1, to 2 years by freezing brot cultures or cell suspensions in suitable vials.

•Deep freezing of microorganisms requires a cryoprotectant such as glycerol or dimethyl sulfoxide (DMSO) when stored at -70°C or in the liquid nitrogen at -156 to -196°C.

•Broth cultures taken in the mid--logarithmic to late logarithmic growth phase are mixed with an equal volume of 10 to 20% (vol/vol) glycerol or 5 to 10% (vol/vol) DMSO.
.Alternatively, a 10% glycerol-sterile broth suspension of growth from agar slants may be prepared.

Preservation in Liquid Nitrogen

Storage in liquid nitrogen is clearly the preferred method for preservation of culture viability.

Protocol for Cryopreservation

•After centrifugation the supernatant is removed and the pellet, consisting of microbial cells, is dissolved in an ice-cold solution containing polyvinyl ethanol (10% [wt/vol]) and glycerol (10% [wt/vol]) in a 1:1 ratio.

•Due to the presence of polyvinyl ethanol, a viscous thick cell suspension is obtained, which is kept for about 30 minutes in an ice bath for equilibration.

•During equilibration, an aliquot of 0.5 to 1.0 ml of the cell suspension is dispensed into each plastic cryovial or glass ampoule.

• They are tightly closed, clamped onto labeled aluminum canes, and placed at -30°C for about 1 h or for a few minutes in the gas phase of liquid nitrogen to achieve a freezing rate of about 1°C/min.

•The canes are then placed into canisters, racks, or drawers and frozen rapidly at -80°C or in liquid nitrogen.

- For revival of cultures, the frozen ampoules are removed from the liquid nitrogen.

- For thawing, they are immediately immersed to the neck in a water bath at 37°C for a few seconds.

- The thawed cell contents of the ampoule or vial are immediately transferred to membranes to form a thick layer.

- The resulting bacterial membranes with immobilized cells are used as a biological component of a biosensor for activity measurements.