Primary and Secondary Screening of Industrially Important Microorganisms

Introduction

Screening is the process of detecting, isolating, and identifying industrially significant microorganisms from a mixed population. This involves both **primary screening** and **secondary screening** techniques.

Primary Screening

Primary screening involves simple techniques to detect and isolate valuable microorganisms based on biochemical characteristics.

Steps in Primary Screening

1. **Sample Collection** – Microbial samples are taken from sources such as soil, water, air, milk, and compost.
2. **Serial Dilution** – Samples are diluted to obtain distinct colonies.
3. **Isolation on Agar Medium** – The diluted sample is spread on agar plates for microbial growth.
4. **Identification of Industrially Important Microbes** – Based on observable characteristics like zone of inhibition.

Types of Primary Screening

1. Screening of Antibiotic-Producing Microorganisms

- **Crowded Plate Technique** – Identifies antibiotic producers by observing zones of inhibition around colonies.
- **Wilkin's Agar Overlayer Method** – Uses high dilutions to isolate antibiotic producers. Wilkin's agar with pH indicators helps differentiate between antibiotic and acid/alkali production.

2. Screening of Organic Acid-Producing Microorganisms

- **pH Indicator Dyes** – Neutral Red and Bromothymol Blue change color based on acid production.
- **Calcium Carbonate Incorporation** – Acid producers form clear zones around colonies due to $CaCO_3$ dissolution.

3. Enrichment Culture Technique

- Used to isolate microorganisms with specific nutrient requirements by incorporating their required nutrients in the medium.
- **Examples:**
  - *Protease producers* – Grown in protein-containing media.

- *Cellulase producers* – Grown in cellulose-containing media.
- *Lysine producers* – Grown in media lacking lysine.

4. Auxanography Technique

- Detects microorganisms producing extracellular metabolites (e.g., vitamins, amino acids).
- **Procedure:**
  - First plate: Microbes grow on nutrient agar.
  - Second plate: A minimal media seeded with test organisms is prepared.
  - The first plate is placed over the second plate.
  - Zones of stimulated growth indicate production of extracellular metabolites.

Secondary Screening

Primary screening helps identify microorganisms with potential industrial applications, but it does not provide details about their production efficiency. Secondary screening is a systematic method to identify industrially significant microorganisms with high yield potential and commercial value.

- The ability of microorganisms to produce industrially relevant metabolites.
- The quality and yield of the produced compounds.
- The type of fermentation process suitable for production.
- The genetic stability and economic feasibility of the microorganism.
- Helps in optimizing growth conditions for maximum yield.
- Identifies the chemical, physical, and biological properties of microbial products.
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Giant Colony Technique

The Giant Colony Technique is a secondary screening method used to isolate and detect antibiotic-producing microorganisms, particularly those that produce antibiotics capable of diffusing through solid media.

Procedure:

1. **Inoculation:**
   - A selected *Streptomyces* species (or other antibiotic-producing microorganisms) is inoculated at the center of a sterilized petri dish containing nutrient agar medium.
2. **Incubation:**
   - The plate is incubated until the *Streptomyces* culture grows sufficiently and starts producing antibiotics.
3. **Introduction of Test Organisms:**
   - Test organisms (potential pathogens or indicator strains) are streaked from the edges of the plate towards, but not touching, the *Streptomyces* colony.
4. **Observation of Growth Inhibition:**
   - The plate is further incubated to allow test organisms to grow.

- The extent to which the test organisms' growth is inhibited by the antibiotics diffusing from the *Streptomyces* culture is measured in millimeters.

5. **Determination of Inhibition Spectrum:**
   - The relative inhibition of different test organisms by the antibiotic is termed the **inhibition spectrum**.
   - Test organisms highly sensitive to the antibiotic exhibit significant growth inhibition.
6. **Selection of Potential Producers:**
   - *Streptomyces* strains that effectively inhibit microbial growth are preserved for further antibiotic production studies.

Advantages of Giant Colony Technique:

- Allows rapid identification of potent antibiotic producers.
- Simple and cost-effective method for screening large numbers of microbial isolates.
- Provides insight into the inhibition spectrum and potential industrial application of microbial metabolites.

Other Secondary Screening Methods

Filtration Method: Used for antibiotics that do not diffuse well through solid media. The Streptomyces culture is grown in liquid broth, filtered, and the filtrate is tested for antibiotic activity using dilution methods.

Liquid Medium Method: Used to quantify antibiotic production. The microorganism is cultured in a highly nutritive liquid medium under continuous aeration and incubation, optimizing metabolite yield.

Comparison: Primary vs. Secondary Screening

Primary Screening	Secondary Screening
Simple isolation techniques	Detailed biochemical and genetic analysis
Identifies potential microbial producers	Evaluates yield, stability, and economic feasibility
Does not provide exact production yield	Determines production potential and stability
Example: Crowded plate method	Example: Chromatographic analysis

Conclusion

Primary screening helps in the initial identification of microorganisms with industrial potential, while secondary screening ensures that the selected organisms have high commercial viability. A combination of these techniques is essential for discovering new industrially significant microorganisms and optimizing their production processes.

Preservation Techniques for Industrially Important Microorganisms

Introduction Preservation refers to maintaining pure cultures of microorganisms in a viable state for extended periods without any genetic change. The main goal is to halt microbial growth or significantly reduce the growth rate to prevent toxic accumulation and contamination while maintaining viability.

Types of Preservation Methods

Microorganism preservation techniques are categorized into:

1. **Short-term Methods**
2. **Long-term Methods**

Short-term Methods

1. Periodic Transfer to Fresh Medium

- Involves regularly subculturing microorganisms onto fresh media.
- Bacteria remain viable for 2–4 weeks, while fungi last 3–4 months.
- Simple and widely used but requires continuous maintenance.

2. Storage in Saline Suspension

- Bacteria are preserved in a 1% salt concentration in screw-capped tubes.
- Storage at room temperature prevents evaporation.
- Culture is revived by transferring it to agar slants.

3. Storage at Low Temperature

- **Refrigeration (4°C):** Slows down microbial metabolism, but toxic byproducts may accumulate over time.
- **Cryopreservation (-10°C to -196°C):** Microorganisms are frozen with stabilizing agents like glycerol (15%) or dimethyl sulfoxide (DMSO) to prevent ice crystal formation.

4. Storage in Sterile Soil

- Mainly used for sporulating fungi such as *Fusarium*, *Penicillium*, and *Rhizopus*.
- Spore suspension is inoculated into autoclaved soil and incubated before refrigeration.
- Can maintain viability for up to 70–80 years.

5. Preservation by Overlaying Culture with Mineral Oil

- Sterile mineral oil is poured over agar slants containing microbes.
- Stored at room temperature or at 0–5°C.

- Reduces oxygen access and limits metabolism, extending viability from 7–12 years.

6. Immersion in Distilled Water

- Used for fungal preservation.
- Cultures immersed in sterile distilled water can remain viable for 2–10 years.

Long-term Methods

1. Cryopreservation

- Microorganisms are rapidly frozen in liquid nitrogen (-196°C) or in the gas phase (-150°C).
- Cryoprotective agents like glycerol and DMSO prevent ice crystal damage.
- Ensures viability for 10–30 years.

2. Lyophilization (Freeze-Drying)

- Microbes are frozen and then dried under a vacuum to prevent ice crystallization.
- Stored in glass vials and sealed for long-term preservation.
- Suitable for spore-forming bacteria (*Bacillus*, *Clostridium*) but not ideal for molds, protozoa, and some viruses.
- Viability maintained for up to 30 years.

Stages of Lyophilization:

1. **Pretreatment** – Sample preparation
2. **Freezing** – Solidification of culture
3. **Primary Drying** – Sublimation (water removal)
4. **Secondary Drying** – Desorption (removes bound water)

Methods:

- **Chamber Method:** Uses double glass vials with silica gel for moisture control.
- **Manifold Method:** Uses single glass vials, sealed to maintain vacuum conditions.

3. Storage in Silica Gel

- Microorganisms are stored in silica gel powder at low temperatures.
- Suitable for *Saccharomyces cerevisiae*, *Aspergillus nidulans*, and *Escherichia coli*.
- Maintains viability for 1–2 years.

Advantages & Disadvantages of Preservation Methods

Method	Advantages	Disadvantages
Periodic Transfer	Simple and cost-effective	Requires frequent maintenance
Saline Suspension	Inexpensive	Short-term viability
Low-Temperature Storage	Easy to maintain	Toxic accumulation over time
Cryopreservation	Long-term viability (10–30 years)	Expensive and requires liquid nitrogen
Lyophilization	Longest shelf life, easy transport	High cost, unsuitable for molds and viruses
Mineral Oil Storage	Long-term viability (7–12 years)	Limited to certain microbes
Sterile Soil Storage	Long-term viability (70–80 years)	Applicable mainly for fungi
Silica Gel Storage	Quick drying, cost-effective	Viability limited to 1–2 years

Conclusion

Selecting an appropriate microbial preservation method depends on the type of microorganism, the required storage duration, and resource availability. While short-term methods are suitable for routine use, long-term techniques like cryopreservation and lyophilization ensure extended viability with minimal genetic alterations.

Culture Repositories for Industrially Important Strains

Culture repositories play a crucial role in preserving and distributing industrially significant microbial strains used in biotechnology, pharmaceuticals, food, and agriculture. These repositories maintain bacteria, fungi, yeasts, and actinomycetes essential for enzyme production, biofuel synthesis, antibiotics, and fermented products.

Major culture collections include:

- **ATCC (USA)** – American Type Culture Collection
- **DSMZ (Germany)** – Deutsche Sammlung von Mikroorganismen und Zellkulturen
- **NCIM (India)** – National Collection of Industrial Microorganisms
- **MTCC (India)** – Microbial Type Culture Collection
- **JCM (Japan)** – Japan Collection of Microorganisms
- **NRRL (USA)** – Northern Regional Research Laboratory (now ARS Culture Collection, USDA)

These repositories ensure strain authenticity, genetic stability, and global accessibility. They provide reference strains for research, patenting, and commercial applications. Cryopreservation and lyophilization techniques help maintain viability. Culture repositories

support innovation by facilitating strain improvement, bioprospecting, and sustainable industrial production of bio-based materials, enzymes, and pharmaceuticals.

Strain Improvement for Industrial Applications

Introduction Strain improvement is the science and technology of manipulating and enhancing microbial strains to increase their metabolic capabilities. The objective is to optimize the production of industrially important metabolites, enzymes, and pharmaceuticals while reducing costs and improving efficiency.

Ideal Characteristics of an Industrial Strain

- Rapid growth and genetic stability
- Non-toxicity to humans
- Ability to utilize cheaper substrates
- Elimination of byproducts that interfere with downstream processing
- Efficient use of carbon and nitrogen sources
- Reduced cultivation costs
- Shorter fermentation times

Approaches for Strain Improvement

1. **Mutant Selection**
2. **Recombination**
3. **Recombinant DNA Technology**

Mutant Selection

A mutation is a sudden and heritable change in an organism's traits. Strains can be improved using mutagenesis, which involves exposing microbes to mutagens to induce beneficial mutations.

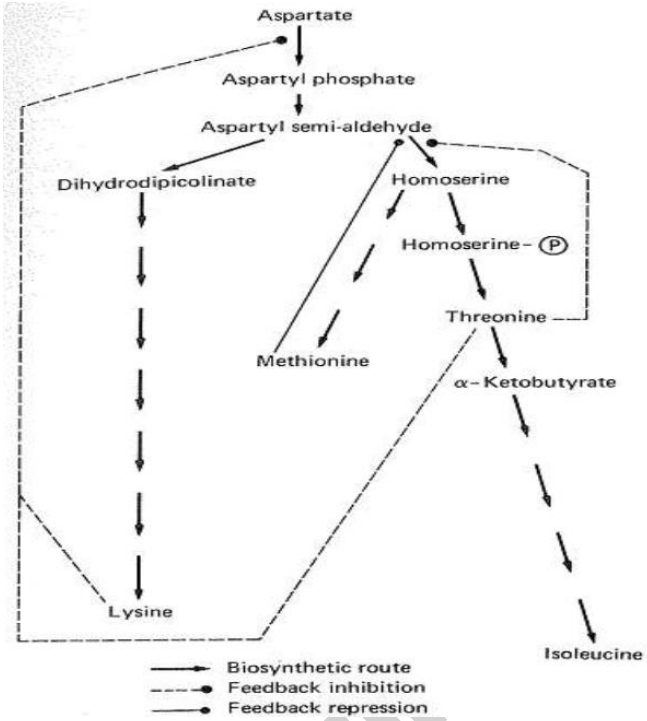
Types of Mutations

- **Spontaneous Mutation:** Occurs naturally without external treatment.
- **Induced Mutation:** Caused by mutagenic agents (physical or chemical).

Mutant Selection Techniques

1. **Isolation of Auxotrophic Mutants**
    - These mutants have defects in biosynthetic pathways and require specific biomolecules for growth.

- Example: *C. glutamicum* Phe⁻ mutant accumulates tyrosine.



2. **Analogue-Resistant Mutants**
   - Possess feedback-insensitive enzymes that enhance production.
   - Example: *C. glutamicum* Tyr⁻ mutant selected for resistance to p-fluorophenylalanine.
3. **Revertants from Non-Producing Mutants**
   - Reversion mutations restore lost metabolic functions, leading to higher yields.
   - Example: *Streptomyces viridifaciens* revertant increased chlortetracycline production 6-fold.
4. **Antibiotic-Resistant Mutants**
   - Selection for resistance to self-produced antibiotics can lead to increased yields.
   - Example: *S. aureofaciens* mutants resistant to chlortetracycline showed 4-fold higher production.
5. **Mutants with Altered Cell Membrane Permeability**
   - These mutants actively excrete metabolites, improving yield.
   - Example: *E. coli* mutant defective in lysine transport excretes high L-lysine levels.
6. **Mutants Producing Altered Metabolites**
   - Some mutants generate novel or improved metabolites.
   - Example: *Pseudomonas aurofaciens* mutant produces 4'-fluoropyrrolnitrin.

Recombination for Strain Improvement

Recombination is the formation of new gene combinations by exchanging genetic material between strains. It is used for genetic analysis and strain enhancement.

Types of Recombination

- **Cross-over Events**
- **Transformation** (Uptake of free DNA from the environment)
- **Conjugation** (DNA transfer via pili between bacteria)
- **Transduction** (Gene transfer via bacteriophages)
- **Protoplast Fusion**
  - Fusion of non-producing strains can yield novel products.
  - Example: *S. griseus* × *S. tenjimariensis* fusion resulted in a strain producing indolizomycin.
- **Parasexual Cycle**
  - Occurs in fungi that lack a sexual stage.
  - Example: *A. niger*, *P. chrysogenum*, and *A. nidulans* demonstrate parasexual recombination.

Recombinant DNA Technology

Recombinant DNA (rDNA) technology involves inserting specific genes into microbes to enhance productivity.

Applications of rDNA Technology

1. **Production of Recombinant Proteins**
   - Example: Insulin and interferons are produced in bacterial hosts.
2. **Metabolic Engineering**
   - Altering metabolic pathways to increase yield.
   - Example: *C. glutamicum* engineered for enhanced isoleucine and ethanol production.
3. **Product Modification**
   - Engineering new enzymes to modify existing products.
   - Example: Conversion of cephalosporin C to 7-amino cephalosporanic acid using *A. chrysogenum*.
4. **Novel Metabolite Formation**
   - Introduction of new biosynthetic pathways into microbes.
   - Example: *E. coli* engineered with *A. eutrophus* genes for polyhydroxybutyrate synthesis.
5. **Enhanced Growth and Substrate Utilization**
   - Example: *E. coli* engineered with *M. methylotrophus* glutamate dehydrogenase to increase carbon conversion from 4% to 7%.

Key Characteristics for Strain Improvement

1. **Selection of Stable Strains**
   - Stability in productivity is crucial for commercial applications.
   - Example: Micrococcus glutamicus mutants selected for stable lysine production.
2. **Selection of Strains Resistant to Infection**

- Resistance to bacterial or viral infections, such as phage infections in bacterial fermentations.
- Example: Selection of phage-resistant bacterial strains.

3. **Selection of Non-Foaming Strains**
   - Reduces contamination risks and loss of product during fermentation.
   - Example: Development of non-foaming commercial microbial strains.
4. **Selection of Strains Resistant to Medium Components**
   - Some medium components may be toxic; resistant strains ensure higher yield.
   - Example: P. chrysogenum mutants resistant to phenylacetic acid.
5. **Selection of Morphologically Favorable Strains**
   - Morphology influences fermentation efficiency, foaming, and broth filtration.
   - Example: Genetic modifications to control filamentous growth in fungi.
6. **Selection of Strains Tolerant of Low Oxygen Tension**
   - Ensures high productivity even under limited oxygen supply.
   - Example: Lysine-producing strains maintaining output under low aeration.
7. **Elimination of Undesirable Byproducts**
   - Some strains produce unwanted byproducts that interfere with product extraction.
   - Example: Elimination of yellow pigment (chrysogenein) in penicillin-producing strains.
8. **Development of Strains Producing New Fermentation Products**
   - Novel metabolic pathways are introduced to produce valuable industrial compounds.
   - Example: Recombinant DNA technology used to produce new bioactive molecules.

Conclusion Strain improvement plays a vital role in industrial microbiology by enhancing the efficiency, stability, and productivity of microbial strains. The application of genetic and molecular methods ensures the development of strains suitable for large-scale production.

Industrial Media & Sterilization

1. Importance of Growth Media in Industrial Microbiology

- Growth media are essential for optimizing microorganism performance and preventing toxic byproducts.
- Must contain essential nutrients: carbon, nitrogen, minerals, growth factors, and water.
- Raw materials should be cost-effective, available, and easily disposable.

2. Types of Industrial Media

- **Culture Maintenance Media:** Preserves industrial strains and reduces genetic variation.
- **Fermentation Media:** Supports large-scale production processes.
- **Growth Media:** Used for microorganism propagation before fermentation.

3. Fermentation Media Formulation

- Requires a carbon source, nitrogen, phosphorus, sulfur, and trace elements.
- Media must be tailored for:
    - **Primary Metabolite Production:** Optimized for microbial growth.
    - **Secondary Metabolite Production:** Conditions shift after initial growth phase.
- Key raw materials include corn steep liquor, molasses, sulfite liquor, and protein hydrolysates.

In industrial microbiology, the selection of appropriate growth media is crucial for optimizing microbial growth and product formation. The key raw materials used in industrial media must provide essential nutrients while being cost-effective, readily available, and easy to process.

4. Criteria for Choosing Raw Materials

When selecting raw materials for industrial media, the following factors should be considered:

- **Cost-effectiveness**: The material should not exceed the selling price of the final product.
- **Availability**: Raw materials must be easily accessible to avoid production delays.
- **Proximity to Production Site**: Reduces transportation costs.
- **Ease of Waste Disposal**: Should not generate excessive waste or require costly disposal methods.
- **Consistent Quality**: Should maintain stable composition for reliable fermentation.
- **Nutritional Composition**: Must provide essential nutrients like carbon, nitrogen, minerals, and vitamins.

Essential Nutrients in Industrial Media

Industrial microbiology media must satisfy microbial nutritional needs, which include:

1. **Carbon Sources**: Provide energy and carbon units for biosynthesis.
    - Examples:
        - **Carbohydrates**: Glucose, sucrose, starch, molasses
        - **Organic Acids**: Acetate, lactate
        - **Alcohols**: Ethanol, methanol
        - **Oils and Fats**: Plant oils, animal fats
2. **Nitrogen Sources**: Essential for protein synthesis.
    - Examples:
        - **Organic Sources**: Corn steep liquor, yeast extract, peptones, protein hydrolysates (casein, soy, fish)
        - **Inorganic Sources**: Ammonium salts (NH4Cl, (NH4)2SO4), urea
3. **Minerals and Trace Elements**: Required for enzyme function and metabolic activities.
    - Examples: Magnesium, calcium, phosphate, sulfate, potassium, iron, zinc
4. **Growth Factors & Vitamins**: Enhance microbial metabolism.
    - Examples: Biotin, riboflavin, niacin, thiamine
5. **Water**: Serves as a solvent and reaction medium.
6. **Buffers and pH Control Agents**: Maintain stable pH during fermentation.
    - Examples: Phosphate buffers, calcium carbonate

7. **Antifoam Agents**: Prevent excessive foaming in bioreactors.
    - Examples: Silicone oils, vegetable oils

Common Raw Materials in Industrial Media

1. **Corn Steep Liquor (CSL)**
    - A byproduct of maize starch extraction.
    - Rich in nitrogen (amino acids, peptides), vitamins, and minerals.
    - Widely used in antibiotic and enzyme production.
2. **Molasses**
    - A byproduct of sugar refining, containing fermentable sugars.
    - Used in ethanol, citric acid, and yeast production.
    - **Blackstrap molasses**: Cheapest and most common.
    - **High-test molasses**: Higher sugar content, preferred for specialized fermentations.
3. **Yeast Extracts**
    - Derived from waste baker's and brewer's yeast.
    - Rich in amino acids, vitamins, and nucleotides.
    - Used for enzyme and antibiotic fermentations.
4. **Sulfite Waste Liquor**
    - A byproduct of the sulfite pulping process.
    - Contains hexose and pentose sugars.
    - Used in microbial growth media after neutralization.
5. **Protein Hydrolysates**
    - Produced by enzymatic or acid hydrolysis of proteins.
    - Examples: Casein hydrolysate, soy protein hydrolysate, fish and whey hydrolysates.
    - Used in fermentation for antibiotics, enzymes, and biofuels.
6. **Peptones**
    - Derived from meat, gelatin, keratin, soy meal.
    - Provide nitrogen and amino acids.
    - Common in large-scale industrial fermentations.
7. **Pharmamedia**
    - A yellow fine powder from cotton-seed embryo.
    - Rich in protein, minerals, and carbohydrates.
    - Used in tetracycline and penicillin production.
8. **Whey**
    - A byproduct of dairy industry.
    - Used in ethanol, lactic acid, and single-cell protein production.

5. Air and Media Sterilization

- Sterilization eliminates microbial contaminants, using physical or chemical methods.
- **Physical Methods:**
    - **Moist Heat (e.g., autoclaving):** Common and effective for industrial applications.
    - **Filtration:** Used for gases and biostatic fluids.
- **Air Sterilization Techniques:**
    - Filtration, heat, electrostatic repulsion, UV light, chemical agents.
    - Essential for aerobic fermentations to prevent contamination.

6. Batch vs. Continuous Sterilization

- **Batch Sterilization:**
  - Conducted in a fermentor with a heating coil or jacket.
  - Limitations: high steam usage, large plant space, increased equipment cost.
- **Continuous Sterilization:**
  - Uses heat exchangers, holding coils, and coolers.
  - Advantages: faster production time, reduced plant space, cost-effective steam usage.

7. Challenges in Industrial Microbiology

- Maintaining optimal media composition for microbial growth.
- Managing foaming issues that can reduce productivity.
- Preventing contamination through efficient sterilization methods.

Conclusion: Understanding industrial microbiology media and sterilization techniques is crucial for optimizing production efficiency and ensuring microbial culture integrity. The choice of raw materials in industrial media directly affects microbial growth, yield, and economic feasibility. By carefully selecting cost-effective, nutrient-rich sources, industrial microbiology ensures efficient large-scale production of biotechnological products.Proper formulation and sterilization methods significantly impact yield, cost, and process sustainability.