SCREENING OF MICROORGANISMS:

A set of highly selective procedures that allow the detection and isolation of microorganisms producing the desired metabolite (having desired properties) included **Primary Screening**. Primary screening should be rapid, inexpensive, predictive, specific but effective for a broad range of compounds and applicable on a large scale. Primary screening is time-consuming and labor-intensive since a large number of isolates have to be screened to identify a few potential ones. However, this possibly is the most critical step since it eliminates the large bulk of unwanted and useless isolates, which are either non-producers or producers of known compounds.

Rapid and effective screening techniques have been devised for a variety of microbial products, which utilize either a property of the product or that of its biosynthetic pathway for detection of desirable isolates. The initial screening is done in plates using agar media. The microorganisms thus selected are subjected to **secondary screening**. The secondary screening differs from the primary screening both with respect to objective and the level of sophistication. A large amount of information regarding the organism as well as the metabolite is obtained here. Several trials are done to optimize the cultural condition for maximizing the product yield.

Some of the tests done are:

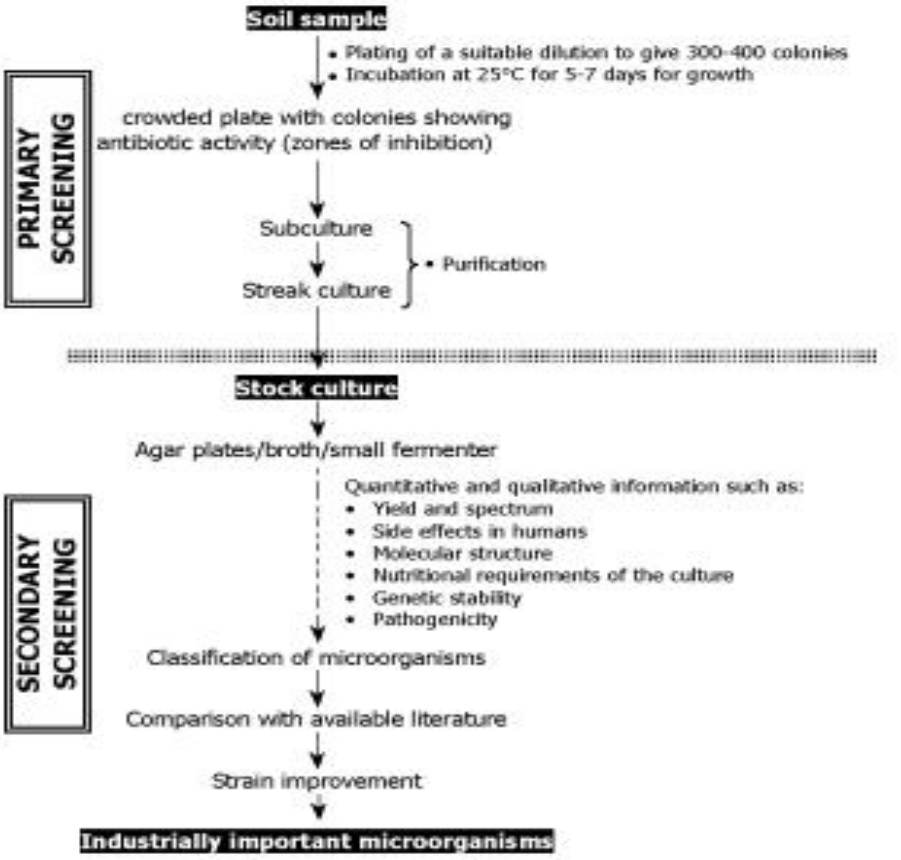
1. For Microorganisms • Classification and identification • Cultural requirements • Pathogenicity • Genetic stability • Scope for improvement, e.g., by mutation, genetic manipulation

2. For Product Yield • Comparison with yield from known commercial strains

3. For Metabolite • Identification of the compound • Immediate or potential use • Toxicity to animals• Novelty (newness)

4. For Process • Shake-flask culture • Pilot-scale culture (inoculum build-up) • Large-scale culture (main fermentation)

Several techniques must be used to all of the above information. The final isolates are often further tested vis-à-vis strain improvement. This final level of selection is termed **selective screening**.



Screening of antibiotic producer from soil

GENERAL TECHNIQUES OF SELECTION OF MICROORGANISMS

The microbial profile of our environment is as diverse as can be, both with respect to type and number. Several selection techniques are available at present to isolate microorganisms of our interest from any environment. Basically, such methods function by facilitating the growth of the desired species so that the subsequent isolation becomes easier. There are three main groups of selection methods:

1- Chemical methods

• Nitrogen or carbon source (for example, cellulose medium for isolating cellulolytic organisms)

• Use of toxic or inhibitory substances (for example media amended with antibiotics, dyes, bile salts, etc.)

2- Physical methods

• Heat treatment: spore-formers can be selected by heating the sample to 80°C for 10 min and culturing.

• Incubation temperature: selection of psychrophiles, thermophiles, etc., is possible by this method.

• pH of the medium: this is especially useful for the isolation of yeasts/molds.

• Cell size and motility: microorganisms can be selected based on size by using filters of varying pore sizes.

3- Biological methods

• Nature also exerts a selective force on microorganisms. Sometimes, animals can serve as a reservoir of a given species of microorganism.

General methods for isolation of microorganisms:

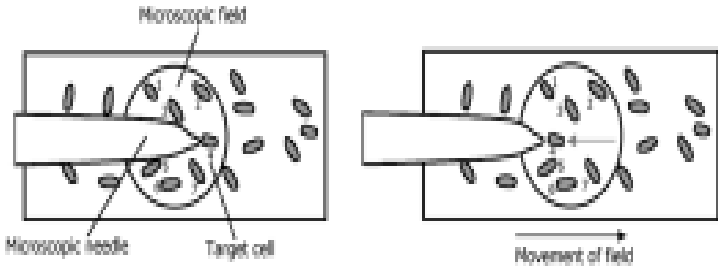
• **Streak-plate technique:** It is a very simple and rapid technique of isolation. In it, the sample broth is streaked onto a dry agar surface. The process thins out the cells and at some point the cells are separated sufficiently apart to give rise to discrete colonies.

• **Pour-plate technique**: It is include mixing of sample broth in a melted agar medium and plating out a suitable dilution. The method has some limitations in that psychrophiles or organisms that cannot withstand a temporary shock of 45-50°C cannot survive. Besides, the isolated colonies remain embedded in the medium and subculturing them entails digging through the agar. The single most important advantage in it is, it can be used for quantitative (enumeration) purpose also.

• **Spread-plate technique:** It involves spreading onto the solid agar surface about 0.1 ml of the culture. In manual method, a loopful of culture is transferred to the agar surface and spread uniformly with a bent glass rod (called dally rod). To affect finer isolation, the residue in the rod is used to spread-plate yet another fresh plate. In this way, 4-6 plates can be used for the spreading.

• **Hypheal tip technique:** This method is useful particularly for isolation of molds. A small segment of mycelia can be aseptically cut out and place on a fresh medium for growth.

• **Micromanipulator technique**: It is used only when clones are required. With this method, a single cell is possible. The method uses an instrument called micromanipulator (a high resolution microscope fitted with manipulating ancillary) and needs considerable expertise. During manipulation, the cell is first identified. Isolation takes place on an agar surface. A sharp needle (provided with the manipulator) is brought close to the cell. The needle is allowed to rest on the agar surface near the cell so that a small dent is formed. Next, the field is shifted away from the needle tip. The cell is later cut out from the agar surface and sub cultured.



Isolation by micromanipulator

PRESERVATION AND MAINTENANCE OF INDUSTRIALLY IMPORTANT CULTURES:

Microorganisms for the production of industrially important products are useful only if they can be maintained indefinitely in healthy, pure, and genetically stable form. Industrial culture collection consists of stock cultures. A stock culture may be simply defined as a culture which serves as a source of inoculum. **Stock cultures** are of two types:

1. Working stock culture: Working stock cultures are maintained in a vigorous and uncontaminated condition and are used frequently. They must be checked frequently for characteristic features and contamination. Primary stock cultures are kept for long-term storage for later use: they are used to produce new working stock cultures as per need.

2. Primary stock culture: As this culture is meant for reserve, it is stored in an uncontaminated state for prolonged periods. The cultures are maintained in a state of low physiological activity. There are several methods available, low risk to high risk. High-risk methods, as evident, use simple methods, often requiring frequent subculturing. Some of the general methods of preservation and maintenance of primary stocks are:

• Periodic transfer, • Oil overlay, • Preservation in soil, • Desiccation or drying, • Lyophilization (freeze-drying), • Use of low temperatures

Periodic transfer method: This method is also called active transfer, subculturing, or serial transfer. The temperature of storage and the type of medium chosen should support a slow, rather than rapid, growth. This reduces the frequency of transfers. Slants (agar slopes) and broth cultures are the most widely used methods. The culture, after maturation, can be preserved in refrigerator and it can be maintained for 2-6 months. Mutation cannot be prevented and the frequent transfers increase the chance of contamination and genetic changes.

Oil overlay method: Many bacteria and other organisms can be preserved by this method for 1 month to 2 years. In this method, an agar slant growth is overlayed with sterile and inert mineral oil and stored cold. The advantage of this method is that some of the growth can be removed to inoculate a fresh medium without contaminating the stock. In other words, the stock can be used for a number of times. This is not possible in subculture method. There are some limitations also, for example, this method causes loss of sporulation, and sometimes, biochemical activity also. Non-sporulating molds, however, can be preserved well. The most common mineral oil used is the liquid paraffin. It is sterilized at 180°C in hot air oven for an hour before using for overlay.

Preservation in soil: Soil can be used either as a carrier or a growth medium. If used as a carrier, abundant spores of bacterial or fungal species must be prepared in advance. The spores are then placed in sterile soil and the resulting preparation dried in air or under vacuum. For many fungi, maintenance by use of soil is very useful. Moist soil may be inoculated with the fungus and may be allowed to grow until sporulation has completed. This type of culture may be used both as primary and as working stock culture.

• Desiccation or drying: This method is useful for preserving yeast cells. Cells are kept directly in contact with silica gel. Silica gel is kept in a sterilized screw-capped container. The culture is grown to a stationary phase in a suitable medium and then

suspended in 5% skim milk medium. The latter is finally transferred to gel without saturating the gel. The gel is dried at room temperature for 2 weeks and finally put in an airtight container for storage at 4°C. The culture is stable for several years.

Lyophilization (freeze-drying) method: Lyophilization is probably the most satisfactory method for long-term preservation of those microorganisms which can withstand the rigor of the process. In this process, a dense cell suspension (in stationary phase) is placed in small vials, frozen under vacuum at –60 to –70°C, and vials sealed using oxygen-natural gas flame. The suspension is prepared in various protective media such as bovine serum, media containing sugars, or skim milk. It is also advantageous to include inositol (5%). The whole is stored under refrigeration in dark. The organism remains viable for as long as 30 years.

The advantages of lyophilization are as follows: • Requires no subculturing • Ease of transportation • Genetic stability • Low cost • Less storage space. The only **disadvantage** of lyophilization is that not all microorganisms can be subjected to this method.

Use of low temperatures: Cells are prepared as a dense suspension in a medium containing a cryoprotectant such glycerol or dimethylsulfoxide. Ampules are prepared and frozen at a controlled rate to –150°C. Initially, the temperature is allowed to go down approximately at the rate of 1°C/min up to –20°C. Thereafter the temperature is brought down as rapidly as is possible. The ampules are stored in liquid nitrogen refrigerator either by immersing (-196°C) or in gas phase (-150°C). This method has been successful with many species, most species remain viable for 20-30 years. The method, however, is very expensive.

SOME CULTURE COLLECTION CENTERS:

• American Type Culture Collection Center (ATCC)

• Commonwealth Mycological Institute (CMI)

• Northern Region Research Laboratory (NRRL)