

**Shrimathi Indira Gandhi College**  
(Nationally Re-accredited at 'A' Grade (3<sup>rd</sup> Cycle) by NAAC)

# SCREENING OF INDUSTRIALLY IMPORTANT MICROORGANISMS

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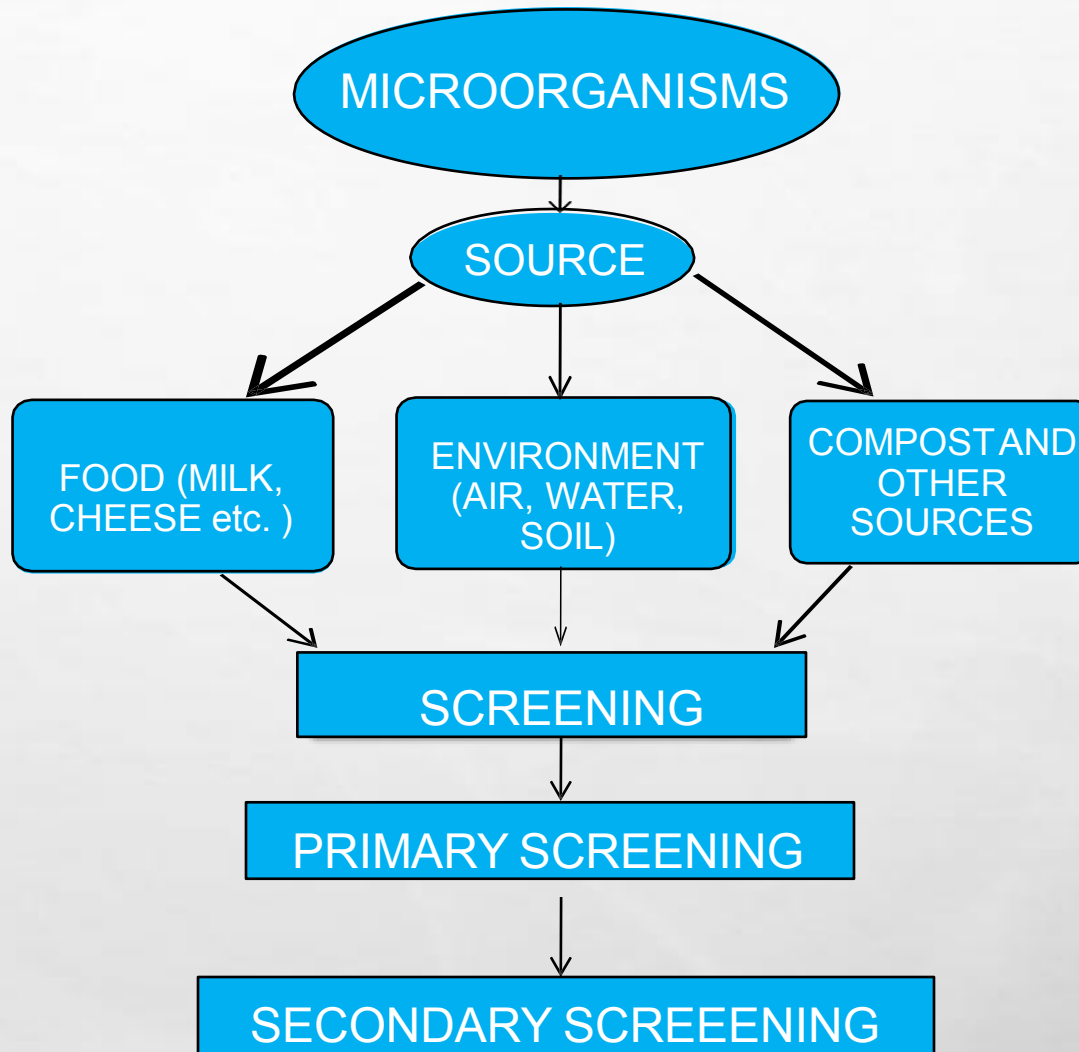
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# SCREENING



The procedure of isolation, detection , and separation of microorganisms of our interest from a mixed population by using highly selective procedures is called **SCREENING**.



## **A producer strain should possess the following characters:**

1. It should be able to grow on relatively cheaper substrates.
2. It should grow well in an ambient temperature preferably at 30-40°C. This reduces the cooling costs.
3. It should yield high quantity of the end product.
4. It should possess minimum reaction time with the equipment used in a fermentation process.
5. It should possess stable biochemical characteristics.

6. It should yield only the desired substance without producing undesirable substances.

7. It should possess optimum growth rate so that it can be easily cultivated on a large scale.

Detection and isolation of a microorganism from a natural environment like soil containing large number of microbial population is called as screening. It is very time consuming and expensive process. For example, Eli Lilly & Co. Ltd discovered three species of antibiotic producing organisms in a span of 10 years and after screening 4,00,000 organisms.

# IMPORTANT THINGS TO BE CONSIDERED WHILE SCREENING:

- 1.) CHOICE OF SOURCE - Samples from screening is taken from soil, water, air, milk, compost etc.
- 2.) CHOICE OF SUBSTRATE -Nutrients and growth factors should be supplied for growth of desired microorganism.
- 3.) CHOICE OF DETECTION - Proper isolation and detection of desired microorganisms is important

# TYPES OF SCREENING

## SCREENING

### PRIMARY SCREENING

### SECONDARY SCREENING

ORGANIC ACID  
PRODUCING  
MICROORGANISMS

ANTIBIOTIC  
PRODUCING  
MICROORGANISMS

EXTRACELLULAR  
METABOLITES  
PRODUCING  
MICROORGANISMS

ENRICHMENT  
CULTURE  
TECHNIQUE

BY USING DYES

BY USING  
CROWDED  
PLATE  
TECHNIQUE

BY  
AUXANOGRAPHY  
TECHNIQUE

BY  
DEFINED  
MEDIA

# PRIMARY SCREENING

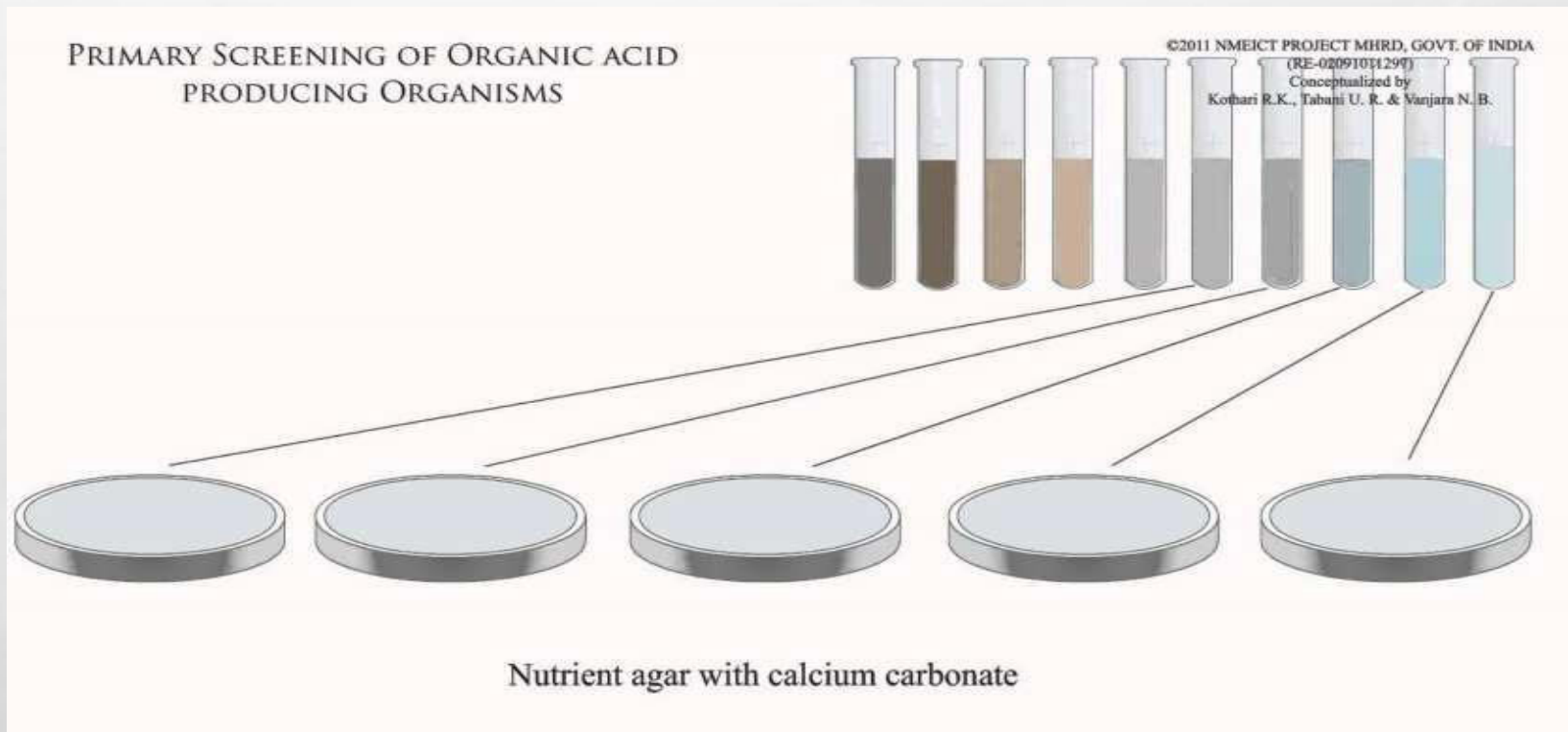
- It's a process for detection and isolation of microorganisms of our interest.
- Determines which microorganisms are able to produce a compounds.
- Does not provide much idea about the production or yield potential of microorganisms.
- It separate out only a few microorganisms, only few have commercial value while discards the valueless microorganisms.



# 1) PRIMARY SCREENING OF ORGANIC ACID PRODUCING MICROORGANISMS

- The pH indicating **dyes** may be used for detecting microorganism that are capable of producing organic acids.
- These dyes undergo color changes according to its pH.
- Dyes such as **Neutral red, Bromothymol blue** are added to the poorly buffered nutrient agar media .
- Colonies are subcultured to make stock culture.
- Further testing is needed since inorganic acids, bases are also metabolic products of microbial growth.

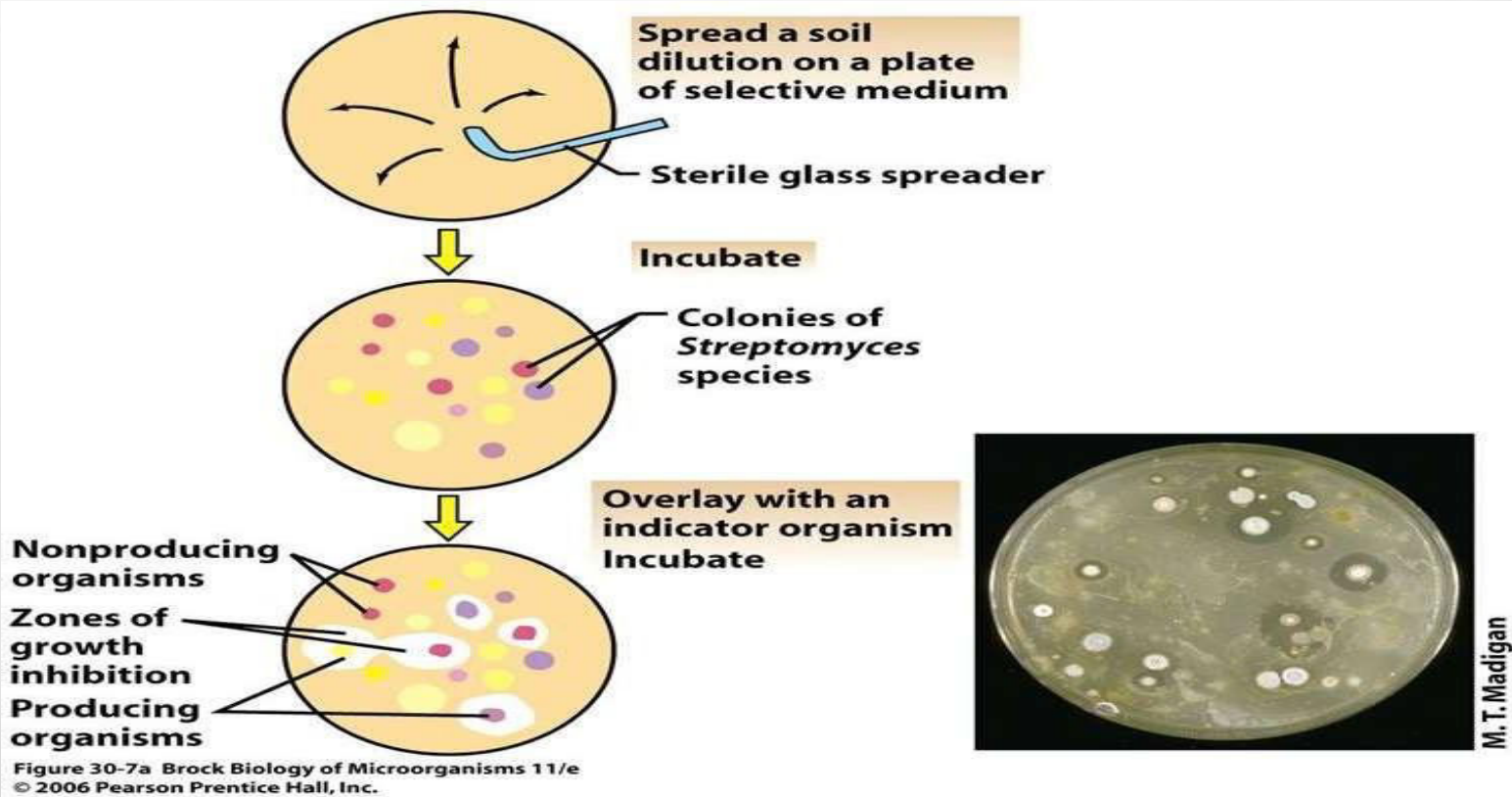
- Incorporation of  $\text{CaCO}_3$  in medium is also used to screen organic acid producing microbes on basis of formation of clear zone of dissolved  $\text{CaCO}_3$  around the colony.



## 2) PRIMARY SCREENING OF ANTIBIOTIC PRODUCING MICROORGANISMS

- ❖ Crowded plate technique is used for screening of antibiotic producing microorganisms.
- ❖ Does not give information about the sensitivity of antibiotics towards other microorganisms.
- ❖ Dilutions are made and then pouring and spreading of soil samples that give 300 to 400 or more colonies per plate.
- ❖ Colonies showing antibiotic activity are indicated by zone of inhibition around the colony .
- ❖ Such colonies are sub cultured and purified by streak before making stock cultures.

- The purified cultures are then tested to find the Microbial Inhibition Spectrum.



### 3) PRIMARY SCREENING EXTRACELLULAR METABOLITE PRODUCING MICROORGANISM

- Auxanography technique is employed for detecting microorganisms able to produce growth factors , vitamins , amino acids etc. extracellularly.
- The 2 major steps are:

A.) Preparation of first plate

- A filter paper strip is put across the bottom of petri dish.
- The nutrient agar is prepared and poured on the paper disc and allowed to solidify.
- Soil sample is diluted and proper dilutions are inoculated.

B.) Preparation of second plate

- A minimal media lacking the growth factors is prepared and seeded with the test organism.
- The seeded medium is poured onto fresh petri plate and the plate is allowed to set.

- The agar in first plate is then lifted and placed on the second plate without inverting.
- The growth factors produced on agar can diffuse into the lower layer containing test organism.
- The zones of stimulated growth of test organism around colonies is an.
- Indication that organism produce growth factor extracellularly.

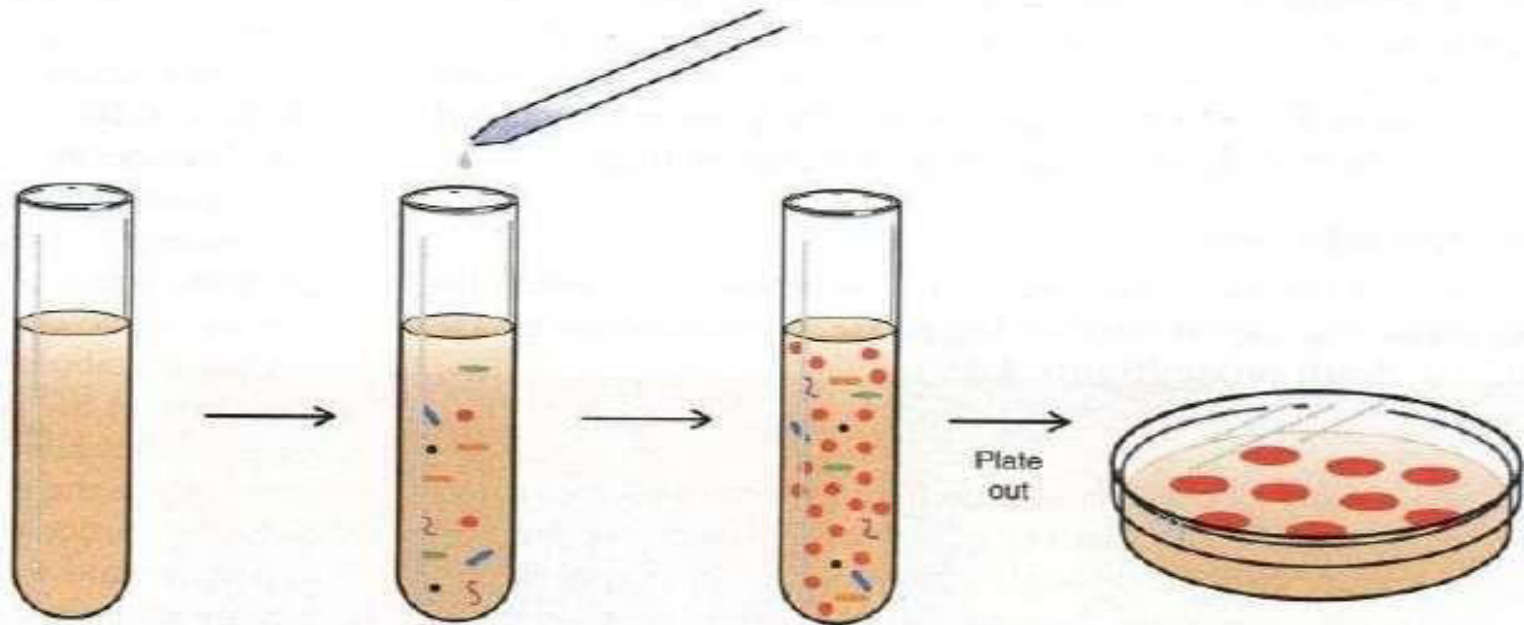


## 4) ENRICHMENT CULTURE TECHNIQUE

- This was designed by Beijerinck to isolate the desired microorganism from heterogeneous microbial population.
- It consists of following steps :
  - a.) Nutrient broth is inoculated with microbial source material and incubated.
  - b.) A small portion of all inoculums is plated onto the solid medium and well isolated colonies are obtained.
  - c.) Suspected colonies from the plate are sub cultured on fresh media and subjected for further testing.

# Enrichment cultures

Isolating an organism from natural sources



Medium contains select nutrient sources chosen because few bacteria, other than the organism of interest, can use them.

Sample that contains a wide variety of organisms, including the organism of interest, is added to the medium.

Organism of interest can multiply, whereas most others cannot.

Enriched sample is plated onto appropriate agar medium. A pure culture is obtained by selecting a single colony of the organism of interest.



# SECONDARY SCREENING

- IT'S A SYSTEMATIC SCREENING PROGRAMME INTENDED TO ISOLATE INDUSTRIALLY IMPORTANT OR USEFUL MICROORGANISMS .

AS PRIMARY SCREENING ALLOWS DETECTION AND ISOLATION OF MICROORGANISMS THAT POSSESS POTENTIALLY INTERESTING INDUSTRIAL APPLICATIONS.

THIS IS USUALLY FOLLOWED BY A SECONDARY SCREENING TO FURTHER TEST THE CAPABILITIES OF AND GAIN INFORMATION ABOUT THESE ORGANISMS.

## **SOME IMPORTANT POINTS ASSOCIATED WITH SECONDARY SCREENING ARE:-**

- It is useful in sorting of microorganisms that have real commercial value. The microorganisms having poor applicability in fermentation process are discarded.
- Provides the information whether the product formed by microorganisms is new or not. This may be accomplished by paper, thin layer, chromatographic technique.

- It should show whether the product possess physical properties such as UV light absorption or fluorescence or chemical properties that can be employed to detect the compound during use of paper chromatography.
- It is conducted on agar plates, in flasks or in small fermentor containing liquid media.
- It gives an idea about the economic position of the fermentation process involving the use of a newly discovered culture.
- It helps in providing information regarding the product yield potentials of different isolates.
- It determines the optimum conditions for growth or accumulation of a product associated with a particular culture.

- Chemical, physical and biological properties of a product are also determined during secondary screening. Moreover, it reveals whether a product produced in the culture broth occurs in more than one chemical form.
- It detects gross genetic instability in microbial cultures. This type of information is very important, since microorganisms tending to undergo mutation or alteration in some way may lose their capability for maximum accumulation of the fermentation products.
- It tells about the chemical stability of the fermentation product.
- It can be qualitative or quantitative in its approach.

- Secondary screening is generally used to obtain information about isolated micro-organism. The information obtained is as follows:-
- It can use to determine qualitative as well as quantitative information about strains. Qualitative in the sense determination of inhibitory spectrum in case of antibiotics and yield potential of the product and quantitative is the determination of the quantity of product obtained by using different fermentation media.
- Secondary screening helps to determine the product produced by fermentation media by using various techniques like chromatography.

- It provides information necessary for classification and identification of organism and due to which the pathogenicity to human and animals can be determined.
- Secondary screening helps to determine the genetic instability of strain. It is very important because if organism carries out mutation and loses the capabilities of giving high yield then the industry may face a great loss.

1. In secondary screening, various nutrients that are provided for growth are tested for toxicity so that any nutrient compound that is toxic to growth of isolated strain should be eliminated.

2. The chemical solubility of product produce is tested. The organic solvents in which the product can get dissolved are eliminated.

3. The physical, chemical and biologically properties of the product are determined and studied.

4. The secondary screening determines the capabilities of an organism to alter or destroy its own product.

Thus secondary screening is all about the collection of a broad range of information about the isolated strain and on the bases of this information, it is decided whether the selected micro-organism is suitable for use on an industrial scale or not.



# EXAMPLE F SECONDARY SCREENING

## ANTIBIOTIC PRODUCING STREPTOMYCES SPECIES

1. Streptomyces isolates are streaked as a narrow band on nutrient agar plates are incubated .
2. Test organisms are then streaked from the edge of plates without touching streptomyceal isolate and then the plates are then incubated .
3. At the end of incubation, growth inhibitory zones for each organism are measured in millimeters .
4. Such organisms are again subjected for further testing by growing the culture in sterilized liquid media and incubated at constant temperature in a mechanical shaker.





Thank  
You...