Isolation & Screening of Microorganisms

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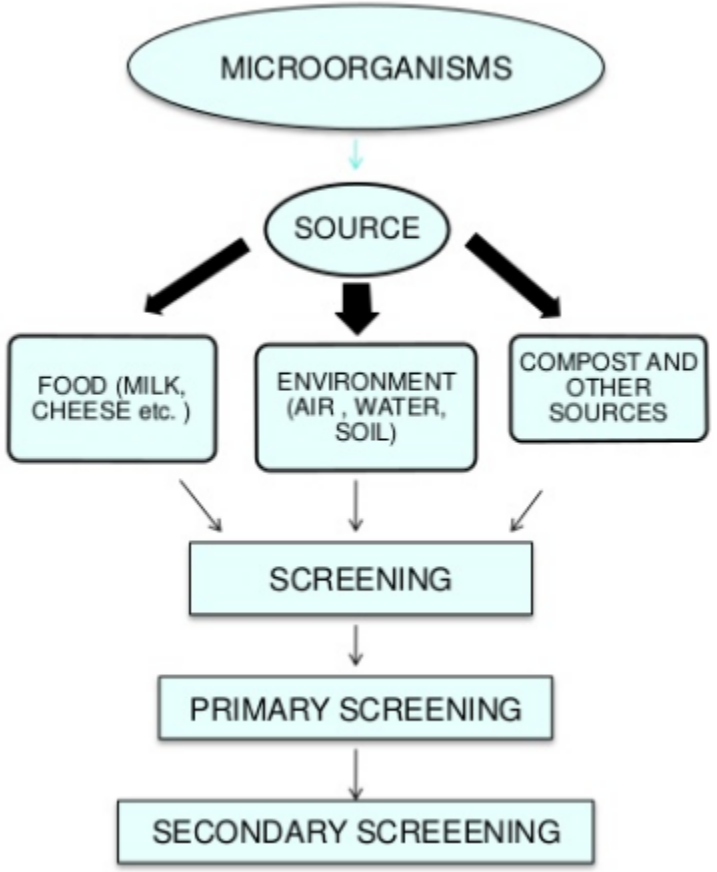
Characteristics of economically important strain

- Pure and free from phage
- Genetically stable, but amenable to genetic modification
- Should produce both vegetative cells and spores
- Should grow vigorously after inoculation in seed stage vessels
- Should produce a single valuable product, and no toxic byproducts
- Product should be produced in a short time, e.g. 3 days
- Should be amenable to long term conservation
- Risk of contamination should be minimal

Isolation of microorganisms

- The first step in developing producer strains is the isolation of concerned microorganisms from the natural habitats.
- The procedure of isolation, detection, and separation of microorganisms of our interest from a mixed population by using highly selective procedures is called **Screening.**



… Isolation of microorganisms

- 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:
- Mos with desired characteristics are found among the natural microflora
- After sampling of the organism the next step is of 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.

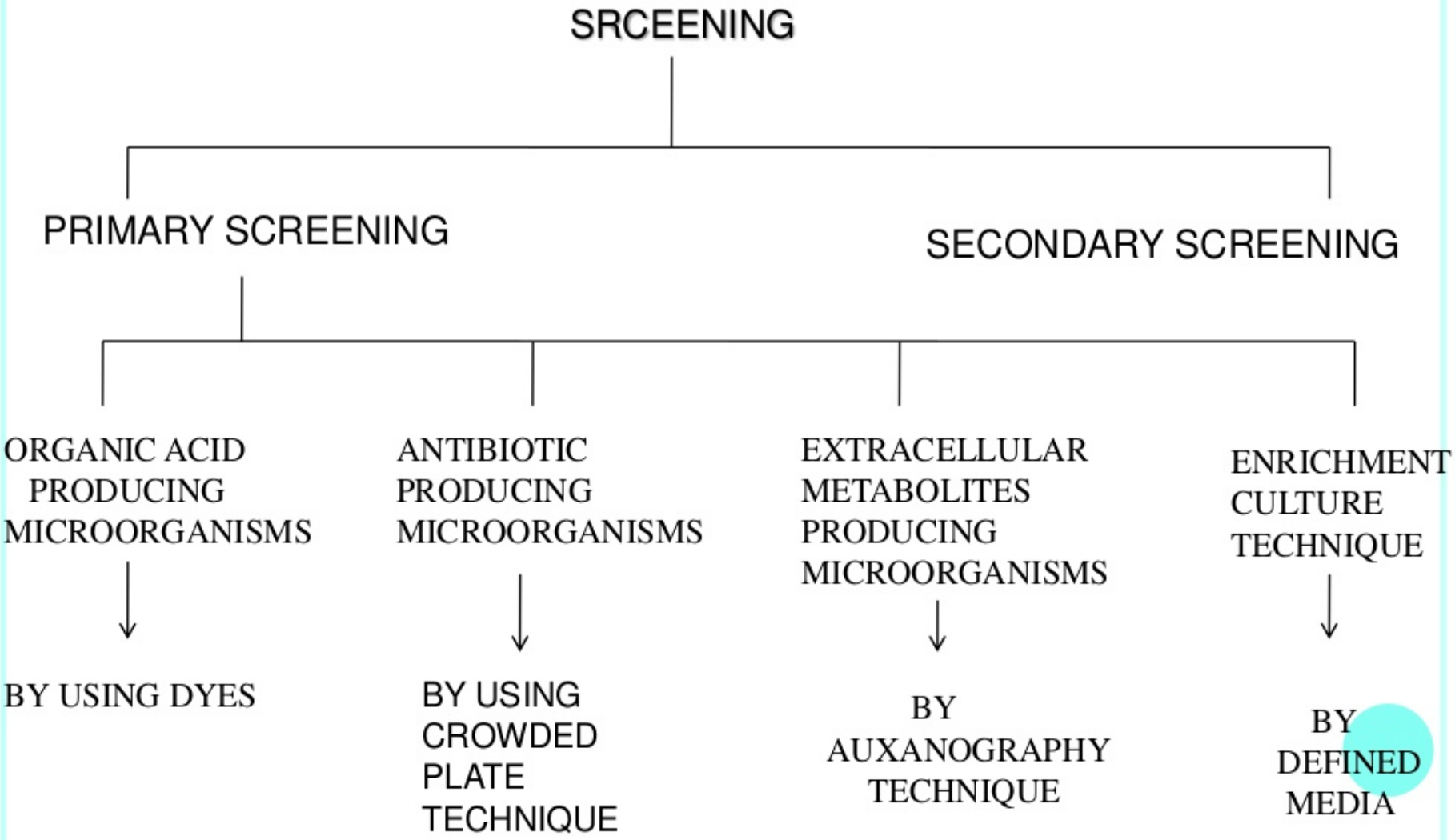
Important thing to be considered while screening

- 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.

Points to be considered:

- 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



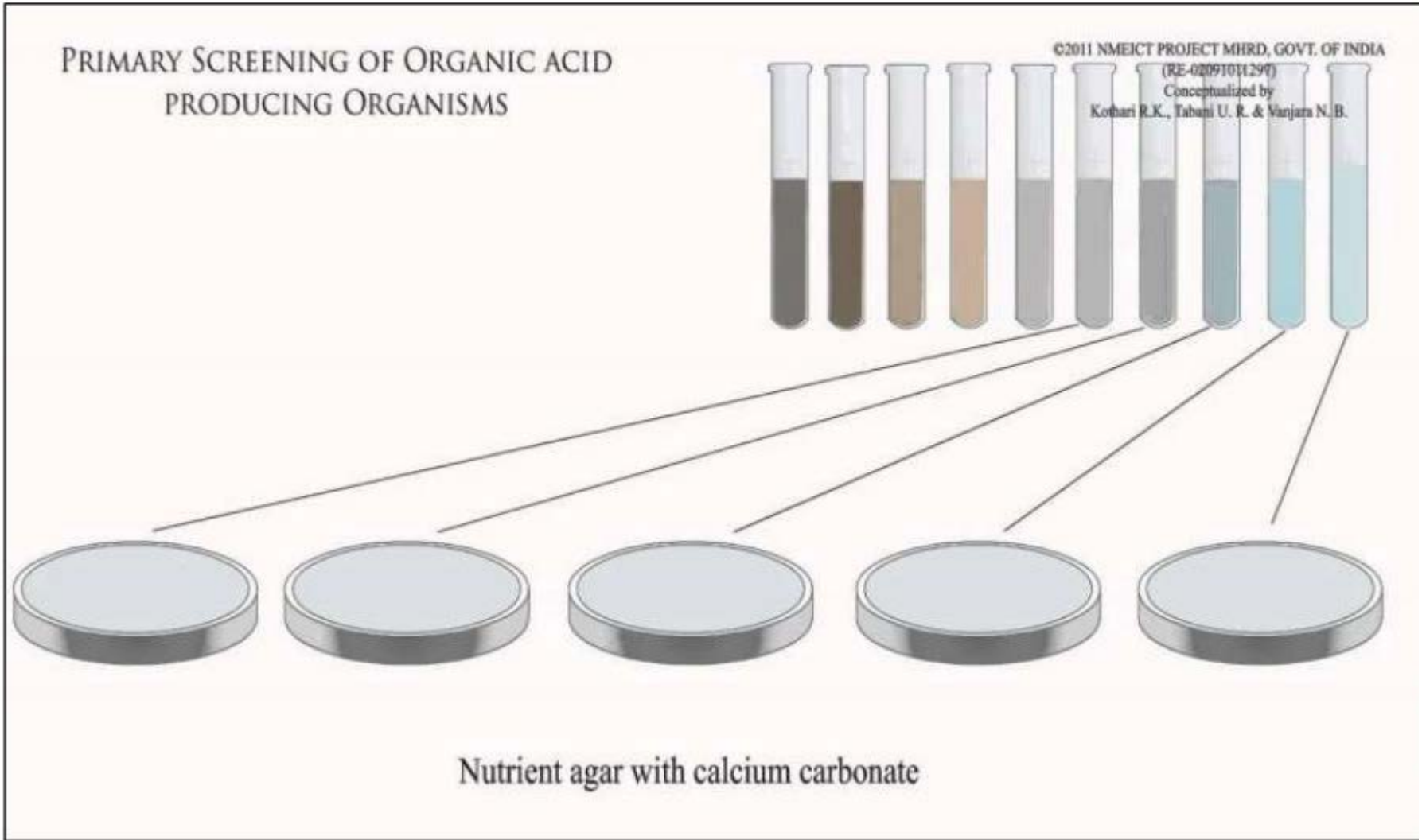
Primary Screening

- A set of highly selective procedures, which allows the detection and isolation of micro-organisms producing the desired metabolite, constitute primary screening.
- It is a time consuming and labour intensive since a large number of isolates have to be screened to identify a few potential ones.
- Rapid and effective screening techniques utilize either:
  - property of the product (eg. For extracellular enzymes)
  - Biosynthetic pathway (eg. Enzyme inhibitors)
- However, for most microbial products of high value, the screening is usually complex and tedious, and often involve two or more steps, eg. For antimicrobials.
- Does not provide much idea about the production or yield potential of microorganisms.

1) PRIMARY SCREENING OF ORGANIC ACID PRODUCING MICROORGANISMS

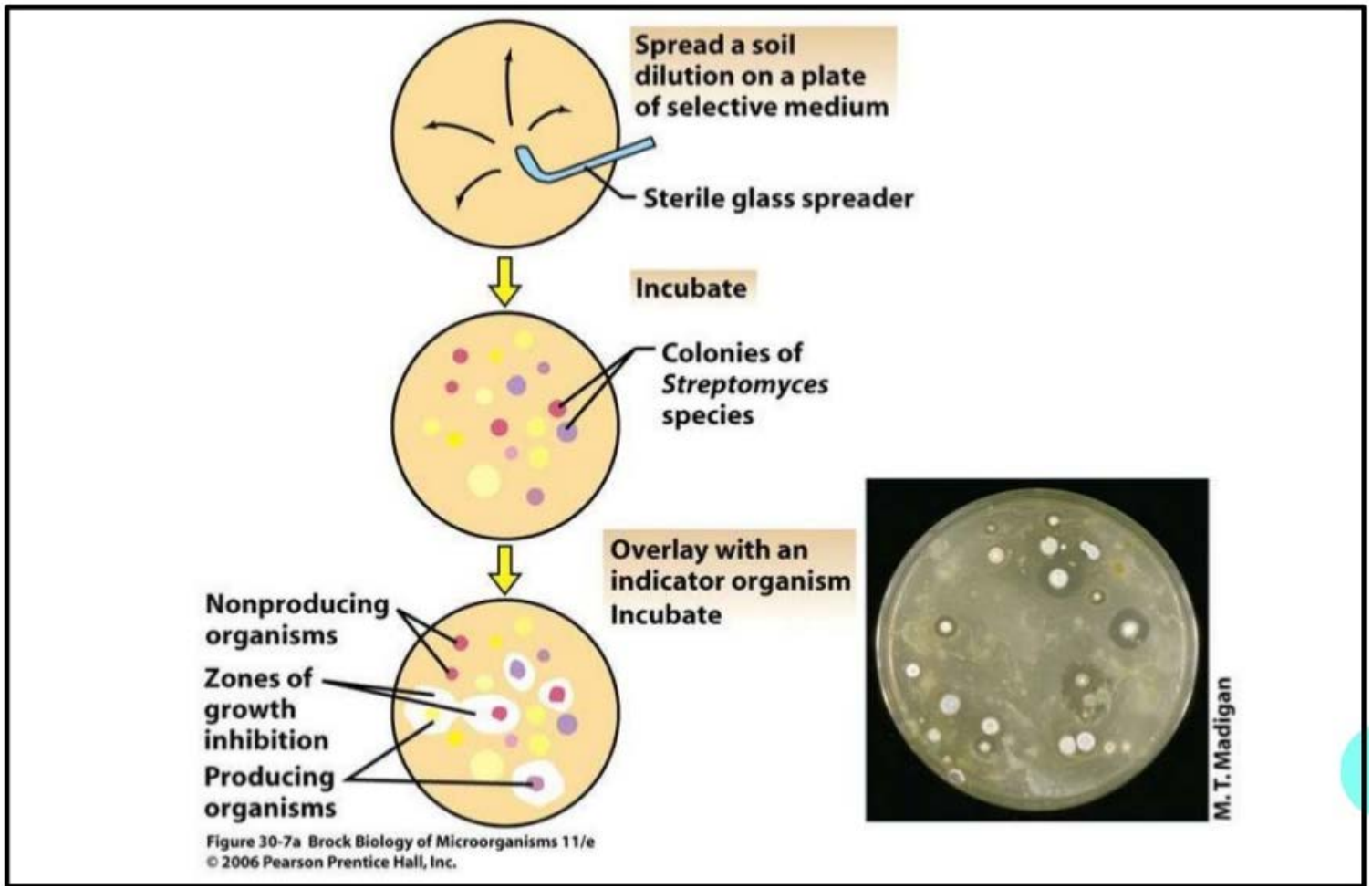
- The pH indicating **dyes** may be used for detecting microorganism that are capable of producing organic acids.
- These dyes undergo color changes according to its pH.
- Dyes such as **Neutral red, Bromothymol blue** are added to the poorly buffered nutrient agar media .
- Colonies are subcultured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

❖ Incorporation of $CaCO_3$ in medium is also used to screen organic acid producing microbes on basis of formation of clear zone of dissolved $CaCO_3$ around the colony.



2) PRIMARY SCREENING OF ANTIBIOTIC PRODUCING MICROORGANISMS

❖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.

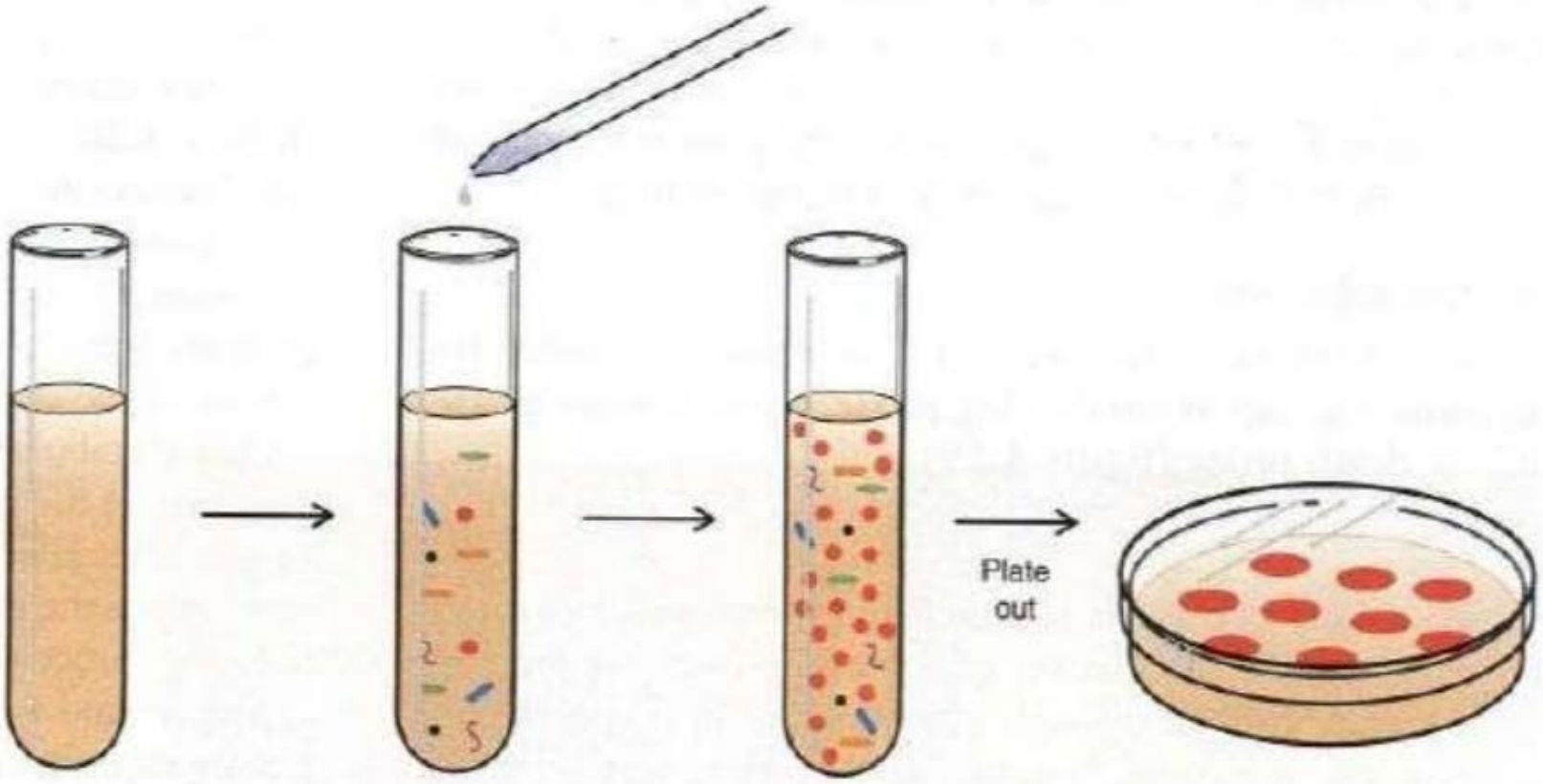
- The agar in first plate is then lifted and placed on the second plate without inverting.
- The growth factors produced on agar can diffuse into the lower layer containing test organism.
- The zones of stimulated growth of test organism around colonies is an indication that organism produce growth factor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

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 is 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.

Questions

- What are the ideal characteristics of industrial strain?
- What are the strategies used for the isolation and screening of industrially important microorganisms?
- What are the screening methods used to detect industrially important microbes?