SEMESTER- VI
INDUSTRIAL MICROBIOLOGY
PAPER XII
COURSE CODE: U-MIB-

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UNIT II: Isolation of industrially important microbial strains and formulation of fermentation media

2.1 Isolation of industrially important microbial strains - Screening Techniques (Primary and secondary), Strain improvement (Basic idea in brief),

2.2. Stock culture and its maintenance (serial subculture, overlaying with mineral oil, lyophilization, liquid nitrogen, soil stock).

2.3. Inoculum development , Fermentation media, (substances used as raw materials for formulation of fermentation media) and its sterilization (batch and continuous).

Screening Techniques

Screening is Isolation and detection of industrially important desired microorganism using highly selective method.

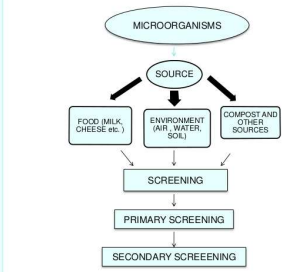
Primary Screening

Secondary Screening

PRIMARY SCREENING	SECONDARY SCREENING
Initial isolation	Further sort out
Large number of isolates obtained	Retain small number
Not confirmed potential	Confirmation of ability of isolate
No detailed study	Strain improvement

SCREENING

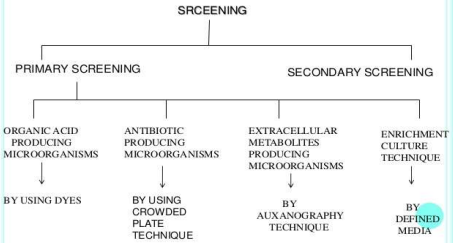
The procedure of isolation, detection , and separation of microorganisms of our interest from a mixed population by using highly selective procedures is called SCREENING



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



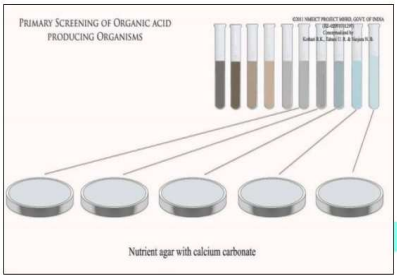
PRIMARY SCREENING

- 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.

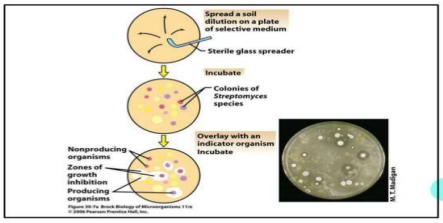
- ❖ Incorporation of $CaCO_3$ in medium is also used to screen organic acid producing microbes on basis of formation of clear zone of dissolved $CaCO_3$ around the colony.



2) PRIMARY SCREENING OF ANTIBIOTIC PRODUCING MICROORGANISMS

- ❖ 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.



Primary screening of antibiotic producers

Crowded plate method

What is antibiotic

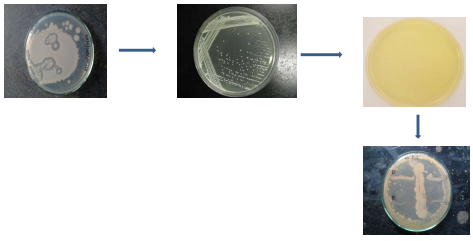
Objective of method

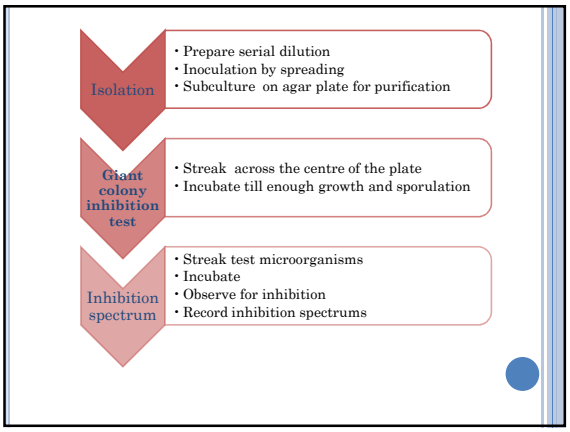
- Microbial source
- Serial dilutions- Select dilution
- Inoculation of plates by spread plate
- Incubation at room temp. 24 hr.
- Select crowded plates
- Select colonies surrounded by clear zone, Zone of inhibition

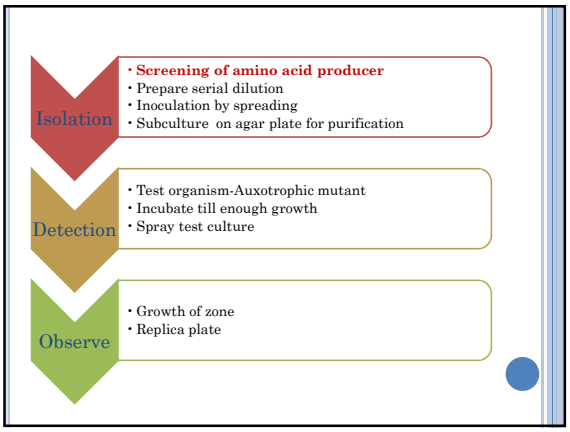
CROWDED PLATE METHOD: (RSML MICRO)

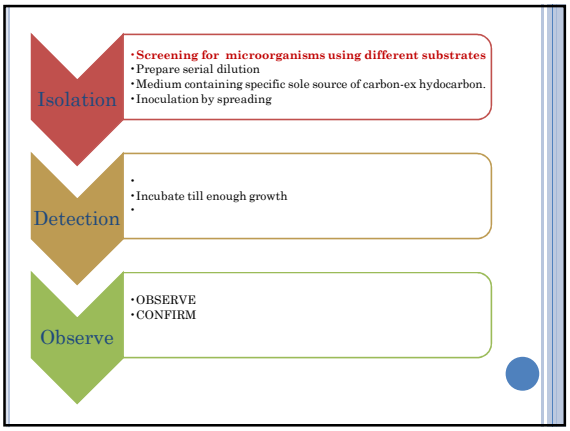
- Limitations of crowded plate method

1. Not give information about type of antibiotic produced
2. Not give information about inhibition spectra
3. It is necessary to confirm whether zone of inhibition is bec. of antibiotic or due to some other reasons









Secondary screening.

Secondary screening gives very useful information about the newly isolated microorganisms that can be employed in fermentation processes of commercial value.

These screening tests are conducted by using petri dish containing solid media or by using flasks or small fermenters containing liquid media.

1. To determine the product produced by an organism is a new compound or not.
2. A determination should be made about the yield potentialities of various isolated microorganisms that are detected in primary screening for that new compound.
3. It should determine about the various requirements of the microorganism such as pH, aeration, temperature etc.
4. It should detect whether the isolated organism is genetically stable or not.
5. It should reveal whether the isolated organism is able to destroy or alter chemically their own fermentative product by producing adaptive enzymes if they accumulate in higher quantities.

It should reveal the suitability of the medium or its constituent chemicals for the growth of a microorganism and its yield potentialities.

7. It should determine the chemical stability of the product.
8. It should reveal the physical properties of the product.
9. It should determine whether the product produced by a microorganism in a fermentative process is toxic or not.

10. Secondary screening should reveal that whether the product produced in fermentation process exists in more than one chemical form. If so, the amount of formation of each chemical formation of these additional products is particularly important since their recovery and sale as byproducts can greatly improve the economic status of the fermentation industry.
11. The new organism should be **identified** to the species level. This will help in making a comparison of growth pattern, yield potentialities and other requirements of test organism with those already described in the scientific and patent literature, as being able to synthesize products of commercial value.

It should select industrially important microorganisms and discard others, which are not useful for fermentation industry.

13. It should determine the economic status of a fermentation process undertaken by employing newly isolated microorganism.

Stock culture maintainance methods

There are a number of reasons why a microbiology laboratory needs stock cultures in good condition. The typical stock culture collection may contain isolates that fall into one or more of the following categories:

1. Reference strains for quality control of culture media and methods
2. Isolates used in the preparation of inoculated samples and specimens for quality control and training purposes
3. Reference strains fosr the development and validation of new methods
4. Pathogens and spoilage organisms lated during routine testing or in the investigation of contamination problems
5. Cultures used in microbiological assays
6. Isolates required for research purposes

There are several National and International CultureCollection Centres. Some of them are given below

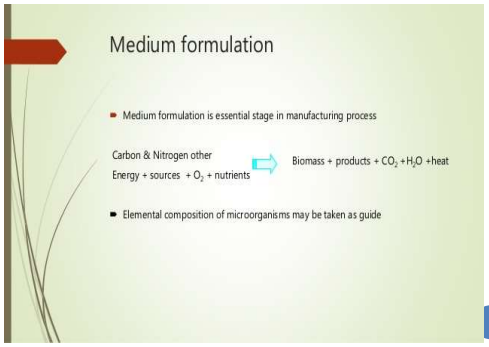
:

1. ATCC (Americsan Type Culture Collection Centre, Maryland, U.S.A.)
2. NCIB (National Collection of Industrial Bacteria, Britain)
3. DSM (Deutsche Sammlung von Mikroorganismen and Zelkulturen, Germany)
4. NCTC (National Collection of Type Culture, London) § MTCC (Microbial Type Culture Collection, Osaka Japan)
5. MTCC (Microbial Type Culture Collection and Gene Bank Institute of Microbial Technology, Chandigarh)
6. ICIM (Indian Culture of Industrial Microorganisms, National Chemical Laboratory, Pune)

RECOMBINATION

- Defined as formation of new gene combinations among those present in different strains.
- Recombination is used for both genetic analysis as well as strain improvement
- To generate new products
- Recombination may be based on:-
  - Transformation
  - Conjugation
  - others like cross over and transduction

- **protoplast fusion** – The fusion between non producing strains of two species (*Streptomyces griseus* and *Streptomyces tenjimariensis*) has yielded a strain that produces indolizomycin, a new Indolizine antibiotic.



- Types of media

Simple media	Complex media
Mineral media for Autotrophs	Ill defined-for Heterotrophs Crude media

- Synthetic and nonsynthetic media

The main factors that affect the final choice of individual raw materials are as follows.

1 Cost and availability: ideally, materials should be inexpensive, and of consistent quality and year round availability.

2 Ease of handling in solid or liquid forms, along with associated transport and storage costs, e.g. requirements for temperature control.

3 Sterilization requirements and any potential denaturation problems.

4 Formulation, mixing, complexing and viscosity characteristics that may influence agitation, aeration and foaming during fermentation and downstream processing stages.

5 The concentration of target product attained, its rate of formation and yield per gram of substrate utilized.

6 The levels and range of impurities, and the potential for generating further undesired products during the process.

7 Overall health and safety implications.

Carbon sources

A carbon source is required for all biosynthesis leading to reproduction, product formation and cell maintenance. In most fermentations it also serves as the energy source.

MOLASSES

1) Pure glucose and sucrose are rarely used for industrial scale fermentations, primarily due to cost.
2) Molasses, a by product of cane and beet sugar production, is a cheaper and more usual source of sucrose.
3) This material is the residue remaining after most of the sucrose has been crystallized from the plant extract.
4) It is a dark coloured viscous syrup containing 50–60% (w/v) carbohydrates, primarily sucrose, with 2% (w/v) nitrogenous substances, along with some vitamins and minerals. It is called black strap molasses
5) Overall composition varies depending upon the plant source, the location of the crop, the climatic conditions under which it was grown and the factory where it was processed.
6) The carbohydrate concentration may be reduced during storage by contaminating microorganisms.
7) A similar product, hydrol molasses, can also be used. This byproduct of maize starch processing primarily contains glucose.

'High test' molasses (*also known as inverted molasses) is a brown thick syrup liquid*

1) used in the distilling industry and containing about 75% total sugars (sucrose and
2) reducing sugars) and about 18% moisture. Strictly speaking, it is not molasses at all but
3) invert sugar, (i.e reducing sugars resulting from sucrose hydrolysis). It is produced by the
4) hydrolysis of the concentrated juice with acid. In the so-called Cuban method, invertase
5) is used for the hydrolysis.

MALT EXTRACT

1) Aqueous extracts of malted barley can be concentrated to form syrups that are particularly useful carbon sources for the cultivation of filamentous fungi, yeasts and actinomycetes.
2) Extract preparation is essentially the same as for malt wort production in beer brewing
3) The composition of malt extracts varies to some extent, but they usually contain approximately 90% carbohydrate, on a dry weight basis. This comprises 20% hexoses (glucose and small amounts of fructose), 55% disaccharides (mainly maltose and traces of sucrose), along with 10% maltotriose, a trisaccharide.
4) In addition, these products contain a range of branched and unbranched dextrins (15–20%), which may or may not be metabolized, depending upon the microorganism.
5) Malt extracts also contain some vitamins and approximately 5% nitrogenous substances, proteins, peptides and amino acids.
6)

WHEY

Whey is an aqueous by product of the dairy industry.

The annual worldwide production is over 80 million tonnes,containing over 1 million tonnes of lactose and 0.2 million tonnes of milk protein.

This material is expensive to store and transport. Therefore, lactose concentrates are often prepared for later fermentation by evaporation of

the whey, following removal of milk proteins for use as food supplements. Lactose is generally less useful as a

fermentation feedstock than sucrose, as it is metabolized by fewer organisms. *S. cerevisiae, for example,* does not ferment lactose.

This disaccharide was formerly used extensively in penicillin fermentations and it is still employed for producing ethanol, single cell protein, lactic acid, xanthan gum, vitamin B12 and gibberellic acid.

SULPHITE WASTE LIQUOR

Sugar containing wastes derived from the paper pulping industry are primarily used for the cultivation of yeasts. Waste liquors from coniferous trees contain 2–3% (w/v) sugar, which is a mixture of hexoses (80%) and pentoses (20%). Hexoses include glucose, mannose and galactose, whereas the pentose sugars are mostly xylose and arabinose. Those liquors derived from deciduous trees contain mainly pentoses. Usually the liquor requires processing before use as it contains sulphur dioxide. The low pH is adjusted with calcium hydroxide or calcium carbonate, and these liquors are supplemented with sources of nitrogen and phosphorus.SCP, *Torula utilis*

Nitrogen sources

Most industrial microbes can utilize both inorganic and organic nitrogen sources.

Inorganic nitrogen may be supplied as ammonium salts, often ammonium sulphate and diammonium hydrogen phosphate, or ammonia.

Ammonia can also be used to adjust the pH of the fermentation.

Organic nitrogen sources include amino acids, proteins and urea.

Nitrogen is often supplied in crude forms that are essentially by products of other industries,

such as corn steep liquor, yeast extracts, peptones and soya meal. Purified amino acids are used only

in special situations, usually as precursors for specific products.

CORN STEEP LIQUOR

- Corn steep liquor is a by product of starch extraction

from maize and its first use in fermentations was for penicillin production in the 1940s.

The exact composition of the liquor varies depending on the quality of the maize and the processing conditions.

Concentrated extracts generally contain about 4% (w/v) nitrogen, including a wide range of amino acids, along with vitamins and minerals.

Any residual sugars are usually converted to lactic acid (9–20%, w/v) by contaminating bacteria.

Corn steep liquor can sometimes be replaced

by similar liquors, such as those derived from potato sstarch production.