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Course Title: Industrial and Food Microbiology

Unit: 3 - Isolation of industrially important strains and Study of fermentation media

Module Name: Primary and Secondary screening

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PRIMARY AND SECONDARY SCREENING

The economics of a fermentation process largely depends upon the type of microorganism used. If fermentation process is to yield a product at a cheaper price the chosen microorganism should give the desired product in a predictable and economically adequate quantity. The microorganism with a desired characters is generally isolated from natural substrates like soil etc.

An industrially important strain should possess the following characters:

1. It should be able to grow on relatively cheaper substrates.
2. It should yield high quantity of the end product.
3. It should possess minimum reaction time with the equipment used in a fermentation process.
4. It should possess stable biochemical characteristics.
5. It should yield only the desired substance without producing undesirable substances.

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. Although there are many screening techniques, all of them are generally grouped into two broad categories.

1. Primary screening, and
2. Secondary screening.

1. Primary Screening of Microorganisms:

Primary screening may be defined as detection and isolation of the desired microorganism based on its qualitative ability to produce the desired product like antibiotic or amino acid or an enzyme etc. In this process desired microorganism is generally isolated from a natural environment like soil, which contains several different species. Sometimes the desired microorganism has to be isolated from a large population of different species of microorganisms.

The following are some of the important primary screening techniques:

(i) The crowded plate technique
(ii) Improved crowded plate technique
(iii) Differential culture technique
(iv) Enrichment culture technique
(v) Auxanographic technique

(i) The Crowded Plate Technique:

This technique is primarily employed for detecting those microorganisms, which are capable of producing antibiotics. This technique starts with the selection of a natural substratum like soil or other source consisting of microorganisms. Progressive serial dilution of the source is made. Suitable aliquot of the serial dilution is chosen which is able to produce 300 to 400 individual colonies when plated on an agar plate, after incubation. Such a plate is called as crowded plate. The antibiotic producing activity of a colony is indicated by no growth of any other bacterial colony in its vicinity. This region of no growth is indicated by the formation of a clear and colorless area around the antibiotic producing microorganism's colony on the agar plate. This region is called as growth inhibitory zone. Such a colony is isolated from the plate and purified either by making repeated sub-culturing or by streaking on a plate containing a suitable medium, before stock culture is made. The purified culture is then tested for its antibiotic spectrum.

(ii) Improved crowded plate technique

Microorganisms capable of producing acids or amines from natural sources can be detected using this method by incorporating certain pH indicator dyes such as neutral red or bromothymol blue into nutrient agar medium. The change in the color of a particular dye in the vicinity of a colony will indicate the ability of that colony to produce an organic acid or base.

(iii) Enrichment Culture Technique:

This technique is generally employed to isolate those microorganisms that are very less in number in a soil sample and possess specific nutrient requirement and are important industrially. They can be isolated if the nutrients required by them is incorporated into the medium or by adjusting the incubation conditions.

(iv) Auxanotrophic Technique:

This technique is employed for the detection and isolation of microorganisms capable of producing certain extracellular substances such as growth stimulating factors like amino acids, vitamins etc. A test organism with a definite growth requirement for the particular metabolite is used in this method.

For this purpose, spread a suitable aliquot on the surface of a sterilized agar plate and allow the growth of isolated colonies, after incubation. A suspension of test organism with growth requirement for the particular metabolite is flooded on the above plate containing isolated colonies, which are subjected to further incubation.

The production of the particular metabolite required by the test organism is indicated by its increased growth adjacent to colonies that have produced the required metabolite. Such colonies are isolated, purified and stock cultures are prepared which are used for further screening process.

(v) Differential culture technique

Production of an organic acid can also be detected by an alternative method. In this method calcium carbonate is incorporated into the agar medium. The production of organic acid is indicated by the formation of a clear zone around those colonies which release organic acid into the medium.

The identified colonies are isolated and purified either by repeated sub-culturing or by streaking methods and a stock culture is made which may be used for further qualitative or quantitative screening tests.

2. Secondary Screening of Microorganisms:

Primary screening helps in the detection and isolation of microorganisms from the natural substrates that can be used for industrial fermentations for the production of compounds of human utility, but it cannot give the details of production potential or yield of the organism. Such details can be ascertained by further experimentation.

This is known as secondary screening, which can provide broad range of information pertaining to the:

1. Ability or potentiality of the organism to produce metabolite that can be used as an industrial organism.
2. The quality of the yield product.
3. The type of fermentation process that is able to perform.
4. Elimination of the organisms, which are not industrially important.

To evaluate the true potential of the isolated microorganisms both qualitative and quantitative analysis are generally conducted. The sensitivity of the test organism towards a newly discovered antibiotic is generally analysed during qualitative analysis, while the quantum yield of newly discovered antibiotic is estimated by the quantitative analysis.

Microorganisms isolated in the primary screening are critically evaluated in the secondary screening so that industrially important and viable potentialities can be assessed.

They include:

1. To determine the product produced by an organism is a new compound or not.
2. It should determine about the various requirements of the microorganism such as pH, aeration, temperature etc.
3. It should detect whether the isolated organism is genetically stable or not.
4. 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.

5. It should reveal the suitability of the medium or its constituent chemicals for the growth of a microorganism and its yield potentialities.
6. It should determine the chemical stability and physical properties of the product.
7. It should determine whether the product produced by a microorganism in a fermentative process is toxic or not.
8. It should select industrially important microorganisms and discard others, which are not useful for fermentation industry.

Methods of Secondary Screening:
Secondary screening gives very useful information pertaining to 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. Each method has some advantages and disadvantages. Sometimes both the methods are employed simultaneously.

There are several techniques and procedures that can be employed for secondary screening. However, only a specific example of estimation of antibiotic substance produced by species of *Streptomyces* is described. Similar methods could be used for the detection and isolation of microorganisms capable of producing other industrial products.

(i) Giant Colony Technique:
This technique is used for isolation and detection of those antibiotics, which diffuse through solid medium. Species of *Streptomyces* are capable of producing antibiotics during primary screening. The isolated Streptomyces culture is inoculated into the central area of a sterilized petri plates containing nutrient agar medium and are selected. The plates are incubated until sufficient microbial growth takes place.
Cultures of test organism, whose antibiotic sensitivity is to be measured are streaked from the edges of plate's up to but not touching the growth of *Streptomyces* and are further incubated to allow the growth of the test organisms. Then the distance over which the growth of different test organisms is inhibited by the antibiotic secreted *Streptomyces* is measured in millimeters.
The relative inhibition of growth of different test organisms by the antibiotic is called inhibition spectrum. Those organisms whose growth is inhibited to a considerable distance are considered more sensitive to the antibiotic than those organisms, which can grow close to the antibiotic. Such species of *Streptomyces*, which have potentiality of inhibiting microorganisms is preserved for further testing.