

Module-6: Primary and Secondary Screening of Industrially Important Microbes

Screening

“The use of highly selective procedures to allow the detection & isolation of only those microorganisms which are of interest from among a large microbial population”

- Screening allows the discarding of many valueless microorganisms, at the same time it allows the easy detection of the useful microorganisms that are present in the population in very less number

Primary screening

“Primary screening allows the detection & isolation of microorganisms that possess potentially interesting industrial application”

- Primary screening separate out only a few microorganisms having real commercial value.
- Primary screening determines which microorganisms are able to produce a compound without providing much idea of the production or yield potential of the organisms

A. Primary screening of organic acid producing microorganisms

- Incorporation of a pH indicating dye such as neutral red or bromothymol blue into a poorly buffered agar medium.
- Greater buffer capacity of medium screen microbes having capability to produce considerable quantities of the acid
- Incorporation of calcium carbonate in the medium is also used to screen organic acid producing microbes on the basis cleared zone of dissolved calcium carbonate around the colony
- These screening approaches do not give idea that which organic acid has been produced
- Thus the colonies of microorganisms showing the potential to produce any fermentation product should immediately be purified and sub-cultured into appropriate medium to be maintained as stock cultures for further testing.

B. Primary screening of antibiotic producing microorganisms

- The simplest screening technique for antibiotic producers is :Crowded Plate” technique
- The technique is used to find out the microorganisms that produce an antibiotic without giving much information of sensitivity towards other microorganisms.
- Procedure include dilution and spreading or pouring of soil samples that give 300 or 400 or more colonies per plate

- Colonies producing antibiotic activity are indicated by an area of agar around the colony
- Such a colony is sub-cultured to a similar medium and purified by streaking, before making stock cultures. The purified culture is then tested to find what types of microorganisms are sensitive in the presence of these the antibiotics i.e. “Microbial Inhibition Spectrum” (MIS).
- The crowded plate procedure also does not necessarily select an antibiotic producing microorganism, because the inhibition area around the colony sometimes can be due to other reason like....

(1) Marked change in the pH of the medium resulted due the metabolism of the colony.

(2) Rapid utilization of critical nutrients in the vicinity of the colony etc.

- Thus further testing is required to confirm the inhibitory activity associated with a microorganisms is whether attributed to the presence of an antibiotic or not
- Screening of antibiotic producing microorganisms can be improved by using a “test organism” and Wilkins method

C. Primary screening of extracellular metabolites (Vitamins, Amino acids and Growth factors) producing microorganisms

D. Primary screening of microorganisms utilizing specific Carbon and Nitrogen sources

Secondary screening

Secondary screening allows further sorting out of microorganisms obtained from PS having real value for industrial processes and discarding of those lacking this potential

1. SS is conducted on agar plates, in flasks or small fermenter containing liquid media
2. SS can be qualitative or quantitative in its approach
3. Secondary screening should give information about the evaluation of the true potential of the microorganisms for industrial usage
4. SS should determine whether microorganisms are actually producing new chemical compounds not previously described
5. SS should reveal whether there is pH, aeration or other critical requirements associated with particular microorganisms, both for the growth of the organism and for the formation of chemical products
6. SS should also detect gross genetic instability in microbial cultures
7. SS should show whether certain medium constituents are missing or possibly, are toxic to the growth of the organisms or its ability to accumulate fermentation products

8. SS should determine whether the product has a simple, complex, or even a macromolecular structure, if this information is not already available
9. SS should show something of the chemical stability of the product and of the product's solubility picture on various organic solvents
10. SS should show whether the product possesses physical properties such as UV light absorption or fluorescence or chemical properties that can be employed to detect the compound during the use of paper chromatography or other analytical methods and which also might be of value in predicting the structure of the compound
11. In some case, for certain kinds of fermentation product determinations should be made as to whether gross animal, plant or human toxicity can be attributed to the fermentation product, particularly if it is utilized (as are antibiotics) in disease treatment
12. SS should reveal whether a product resulting from a microbial fermentation occurs in the culture broth in more than one chemical form and whether it is an optically or biologically active material
13. SS should reveal whether the microorganisms are able to chemically alter or even destroy their own fermentation products
14. Secondary screening helps in predicting the approaches to be utilized in conducting further research on the microorganisms and its fermentation processes.

Strategies for isolation of industrially important microbes

- The diversity of microorganisms may be exploited still by searching for strains from the neutral environment able to produce products of commercial value
- The first stage in the screening of microorganisms of potential industrial is their "isolation"
- Isolation involves obtaining either pure or mixed cultures followed by their assessment to determine which carry out the desired reaction or produce the desired product
- In some cases it is possible to design the isolation procedure in such a way that the growth of producers is encouraged or that they may be recognized at the isolation stage, whereas in other cases organisms must be isolated and producers recognized at a subsequent stage
- It should be remembered that the isolate must carry out the process economically and therefore the selection of the culture to be used is a compromise between the productivity of the organism and the economic constraints of the process.

Criteria used for choice of organisms

- The nutritional characteristics of the organism: Organism should be capable to utilize the ingredients present in the medium to produce interested product.

- The optimum temperature of the organisms: For instance, the use of an organism having an optimum temperature above 40° C considerably reduces the cooling costs of a large-scale fermentation, and therefore, the use of such a temperature in the isolation procedure may be beneficial
- The reaction of the organism with the equipment to be employed
- The stability of the organism and its amenability to genetic manipulation
- The productivity of the organism, measured in its ability to convert substrate into product and to give a high yield of product per unit time.
- The easy product recovery from the cultures.
- It should be a high yielding strain
- It should have stable biochemical characteristics
- It should not produce undesirable substances
- It should be easily cultivated on a large scale
- The ideal isolation procedure commences with an environmental source (frequently soil), which is highly profitable to be rich in the desired types
- Selective pressure may be used in the isolation of organism that will grow on particular substrates in the presence of certain compounds or under agricultural conditions adverse in their types
- If it is not possible to apply selective pressure for the desired character it may be possible to design a procedure to select for a microbial taxon which is known to show the characteristics at a relatively high frequency. E.g. the production of antibiotic by Streptomycin.
- Alternately, the isolation procedure may be designed to exclude certain microbial “weeds” and to encourage the growth of more novel types
- The advantages in the taxonomic description of taxa have allowed the rational design of procedures for the isolation of strains that may have a high probability of being productive or are representatives of unusual groups.
- The advances in pharmacology and molecular biology have also enabled the design of more effective screening tests to identify productive strains amongst the isolated organisms.

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