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Selection of Industrial Microorganisms

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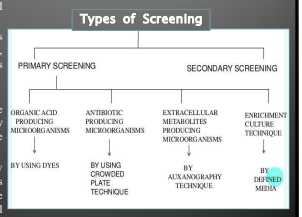
SCREENING

- In Microbial Technology Microorganisms holds the key to the success or failure of a fermentation process.
- It is therefore important to select the most suitable microorganisms to carry out the desired industrial process.
- Detection and isolation of high-yielding ry is of species form the natural sources material, such as soil, containing a heterogeneous microbial population is called Screening.

OR

Screening may be defined as the use of highly selective procedures to allow the detection and isolation of only those microorganisms of interest from among a large microbial population.

- Thus to be effective, screening must, in one or a few steps allow the discarding of many valueless microorganisms, while at the same time allowing the easy detection of the small percentage of useful microorganisms that are present in the population.
- During screening programs except crowded plate technique a natural source such as soil is diluted to provide a cell concentration such that aliquots spread, sprayed or applied in some manner to the surface of the agar plates will yield well isolated colonies (30-300).



Characteristics of Industrial Microorganisms

- **Microorganisms** including bacteria, fungi, actinomycetes and viruses possess some unique qualities different from animals and plants which warrants there usage for most of the industrial processes that produce goods and services of huge economic importance.
- The microorganisms used for industrial productions are usually categorized as **GRAS** (***Generally Regarded As Safe***); and this is because some of these microbes are naturally non-pathogenic and their end-product are usually free from toxic substances.
- Criteria for selection of microbes in biotechnological/industrial microbiology processes are as follows:
- **1. Ability to grow in simple growth medium**: Microorganisms used in industrial microbiology must be able to grow in simple growth medium to maximize profit and cut the cost of adding additional growth nutrients to the medium.
- **2. Production of non-toxic end products:** Since most microbial products are intended for internal use, it is vital that microorganisms used for industrial processes do not produce toxic or undesirable products that generally affect the health of the end users.
- **3. Ability to grow fast**: Microorganisms meant for industrial microbiology processes should be able to grow vigorously and rapidly in the growth medium because slow-growing microbes could impact on the cost of production no matter how efficient the organism may be in terms of production and may cause possible contamination of the production process.
- **4. Ability to produce the desired end product**: The microorganisms used for industrial processes should be able to produce its desired end product under a short period of time in order to avoid contamination and to maximize profit.
- **5. Amenability to genetic manipulations**: The microorganisms should be amenable to genetic manipulations in order to produce or get improved strains of the same organism with better qualities to ensure continued production of the desired end product with improved properties and higher product yield.
- **6. Ability to be resistant to microbial killers:** The microorganisms should be resistant to microbial killers such as bacteriophages and other biotic or abiotic materials/substance that may affect its growth.
- **7. Ability to be genetically and physiologically stable**: The microorganisms should be genetically and physiologically stable i.e. they should not mutate easily. Undesired mutations lead to the production of undesired end products affecting product yield and thus wastage of raw materials used for production.
- **8. Easy to extract of end products**: Microorganisms meant for industrial production should be able to lend itself to a suitable and sustainable method of product recovery.
- **9. Ability to utilize less amount of oxygen**: The microorganisms should have less demand for oxygen or aerobic environments since aeration in the fermenter contributes significantly to the cost of production and that of the end product as well.

Common Industrial Microbes and their Products

Microbial genera/species	Products/usage
Penicillium, Streptomyces, Bacillus	Antibiotics
Recombinant *E. coli, S. cerevisiae*	Insulin
Claviceps purpura	Alkaloids
Rhizopus, Arthrobacter	Steroids
S. cerevisiae	Ethanol
Clostridium acetobutylicum	Acetone & Butanol
Mucor, Aspergillus, Bacillus	Enzymes
Corynebacterium glutanicum	Amino acids
Aspergillus niger	Organic acids
Corynebacterium glutanicum	Nucleotides
Methanobacterium	Methane
Zymomonas, Thermoanaerobacteria	Ethane
Photosynthetic microbes	Hydrogen
Lactobacillus bulgaricus	Yoghurt production

Primary Screening of Organic acid/ Amine Producer

1. For primary screening of organic acid or organic amine producers, soil sample is taken as a source of microorganism.
2. It is diluted serially to an extent to get well-isolated colonies on the plate when spread or applied in some form.
3. After preparation of dilution these dilutions are applied on a media incorporated with a pH indicating dye such as Neutral red (Pink to yellow)or Bromothymol blue (Yellow -blue), into a poorly buffered agar nutrient medium.
4. The production of these compounds is indicated by a change in the color of the indicating dye in the close vicinity of the colony to a color representing an acidic or alkaline reaction.

- The usefulness of this procedure is increased if media of greater buffer capacity are utilized so that only those microorganisms that produce considerable quantities of the acid or amine can induce changes in the color of the dye.
- An alternative procedure for detecting organic acid production involves the incorporation of calcium carbonate (1-2 %) in the medium so that organic acid production is indicated by a cleared zone of dissolved calcium carbonate around the colony.
- These procedures are not error proof, however, since inorganic acids or bases also are potential products of microbial growth. For instance, if the nitrogen source of the medium is the nitrogen of ammonium sulfate the organism may utilize the ammonium ion, leaving behind the sulfate ion as sulfuric acid, a condition indistinguishable form organic acid production.
- Thus cultures yielding positive reactions require further testing to be sure that an organic acid or base actually has been produced.

Primary Screening of Antibiotic Producer (Crowded Plate Technique)

- It consists of preparing a series of dilution of the source material for the antibiotic producing microorganisms, followed by spreading the dilution on the agar plates.
- The agar plates having 300- 400 or more colonies per plate after incubation for 2-4 days are observed.
- Colonies showing antibiotic activity are indicated by the presence of a zone of inhibition surrounding the colony.
- Such a colony is sub- cultured to a similar medium and purified.
- It is necessary to carry on further testing to confirm the antibiotic activity associated with a microorganism since zone of inhibition surrounding the colony may sometimes be due to change in the pH value of the medium resulting from the metabolism of the colony, or rapid utilization of critical nutrients in the immediate vicinity of the colony.
- The crowded plate technique has limited application, since usually we are interested in finding a microorganism producing antibiotic activity against specific microorganism and not against the unknown microorganism that were by chance on the plate in the vicinity of an antibiotic producing organism.
- Antibiotic screening is improved,by the incorporation into the procedure of a "Test organism" that is an organism used as an indicator for the presence of specific antibiotic activity.
- Dilutions of soil or of other microbial sources are applied to the surface of agar plates to obtain well isolated colonies.
- The plates are incubated until the colonies are a few millimeters in diameter and so that antibiotic production will have occurred for those organisms having this potential.
- A suspension of test organism is then sprayed or applied in some manner to the surface of the agar and the plates are further incubated to allow growth of the test organism.
- Antibiotic activity is indicated by zones of inhibited growth of the organism around antibiotic producing colonies.
- Antibiotic producing colonies again must be isolated and purified before further testing.

Primary Screening of Growth Factor (Amino acid/ Vitamin) Producer (Auxanography)

This technique is largely employed for detecting microorganisms able to produce growth factors (eg. Amino acid and Vitamins) extracellularly. The two major steps are as follows:

Step I-

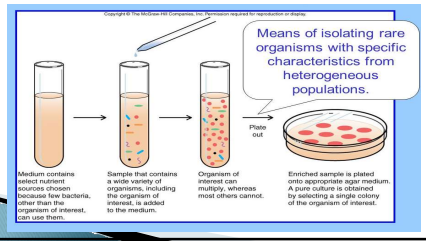
- A filter paper strip is kept across the bottom of a petridish in such a way that the two ends pass over the edge of the dish. A filter paper disc of petridish size is placed over paper strip on the bottom of the plate. The nutrient agar is poured on the paper disc in the dish and allowed to solidify. Microbial source material such as soil, is subjected to dilution such that aliquots on plating produce well isolated colonies. Plating of aliquots of diluted soil sample is done.

Step II-

- A minimal medium lacking the growth factor under consideration is seeded with the test organism. The seeded medium is poured on the surface of a fresh petri dish and allowed to solidify. The agar in the first plate as prepared in step- I is carefully and aseptically lifted out with the help of tweezers and a spatula and placed without inverting on the surface of the second plate as prepared in the second step. The growth factor(s) produced by colonies present on the surface of the first layer of agar can diffuse into the lower layer of agar containing the test organism. The zone of stimulated growth of the test organism around the colonies is an indication that they produce growth factor(s) extracellularly. Productive colonies are sub cultured and are further tested.
- A similar screening approach can be used to find microorganisms capable of synthesizing extracellular vitamins, amino acids or other metabolites. However, the medium at makeup must be totally lacking in the metabolite under consideration. Again the microbial source is diluted and plated to provide well-isolated colonies and the test organism is applied to the plates before further incubation. The choice of the particular test organism to be used is critical. It must possess a definite growth requirement for the particular metabolite and for that metabolite only, so that production of this compound will be indicated by zones of growth or at least increased growth of the test organism adjacent to colonies that have produced the metabolite.

Enrichment Culture Technique

- This technique was designed by Beijerinck, to isolate the desired microorganisms form a heterogeneous microbial population present in soil.
- Either medium or incubation conditions are adjusted so as to favour the growth of the desired microorganism.
- On the other hand, unwanted microbes are eliminated or develop poorly since they do not find suitable growth conditions in the newly created environment.
- Today this technique has become a valuable tool in many screening program for isolating industrially important strains.



SECONDARY SCREENING

- Secondary screening is strictly essential in any systematic screening programme intended to isolate industrially useful microorganisms, since primary screening merely allows the detection and isolation of microbes that possess potentially interesting industrial applications.
- Secondary screening helps in detecting really useful microorganisms in fermentation processes. This can be realized by a careful understanding of the following points associated with secondary screening:

1. It is very useful in sorting our microorganisms that have real commercial value from many isolates obtained during primary screening. At the same time, microbes that have poor applicability in a fermentation process are discarded.
2. It provides information whether the product produced by a microorganism is a new one or not. This may be accomplished by paper, thin layer or other chromatographic techniques.
3. It gives an idea about the economic position of the fermentation process involving the use of a newly discovered culture.
4. It helps in providing information regarding the product yield potentials of different isolates. Thus this is useful in selecting efficient cultures for the fermentation processes.
5. It determines the optimum conditions for growth or accumulation of a product associated with a particular culture.
6. It provides information pertaining to the effect of different components of a medium. This is valuable in designing the medium that may be attractive so far as economic consideration is concerned.
7. It detects gross genetic instability in microbial cultures, since microorganisms tending to undergo mutation or alteration is some way may lose their capability for maximum accumulation of the fermentation products.
8. It gives information about the number of products produced in a single fermentation. Additional major or minor products are of distinct value, since their recovery and sale as by-products can markedly improve the economic status of the prime fermentation.
9. Information about the solubility of the product in various organic solvents is made available.
10. Chemical, physical and biological properties of a product are also determined during secondary screening.
11. It reveals whether the culture is homofermentative or heterofermentative.
12. Determination of the structure of product is done. The product may have a simple, complex or even a macromolecular structure.
13. Determination of the toxicity for animals, plants or man is possible if the products are to be used for therapeutic purpose.
14. It reveals whether microorganisms are capable of chemical change or of even destroying their own fermentation products. E.g. microorganism may produce the adaptive enzyme, decarboxylase can remove carbon dioxide from amino acid, leaving behind an organic amine.
15. It tells us something about the chemical nature of the fermentation product.

SECONDARY SCREENING PROCEDURE

- Thus, secondary screening gives answers to many questions that arise during final sorting out of industrially useful microorganisms.
- This is accomplished by performing experiments on agar plates, in flasks or small bioreactors containing liquid media, or a combination of these approaches.
- A specific example of antibiotic producing Streptomyces species may be taken for an understanding of the sequence of events during a screening programme.
- Those streptomycetes able to produce antibiotics are detected and isolated in a primary screening programme.
- These streptomycetes exhibiting antimicrobial activity are subjected to an initial secondary screening where their inhibition spectra are determined. A simple "Giant – Colony technique" is used to do this.
- Each of the streptomycal isolates is streaked in a narrow band across the centers of the nutritious agar plates. Then, these plates are incubated until growth of a streptomycete occurs.
- Now, the test organisms are streaked from the edges of the plates not touching the streptomycete growth.
- Again, the plates are incubated. At the end of incubation, growth inhibitory zones for each test organism are measured in millimeters.
- Ultimately, streptomycete isolates that have exhibited interesting microbial inhibition spectra need further testing.
- Further screening is carried our employing liquid media in flask, since such studies give more information than that which can be obtained on agar media.
- At the same time, it is advisable to use accurate assay technique (e.g. paper disc agar diffusion assay) to exactly determine the amounts of antibiotic present in samples of culture fluids.
- These streptomycete cultures are inoculated into sterilized liquid media. Then , such seeded flasks are incubated at a constant temperature.
- Moreover, such flasks are agitated by keeping them on mechanical shaker, since the growth of streptomycetes and production of antibiotics occur better in agitated flasks than in stationary ones.

SECONDARY SCREENING PROCEDURE (contd.)

- Samples are withdrawn at regular intervals under aseptic conditions and are tested in a quality control laboratory. Important tests to be carried out include:
  - i. Checking for contamination,
  - ii. Checking of pH
  - iii. Estimation of critical nutrients
  - iv. Assaying of the antibiotic, and
  - v. Other determinations, if necessary
- The result of the above test, points out the best medium for antibiotic formation and the stage at which the antibiotic yields are greatest during the growth of culture on different media.
- After performing all necessary routine tests in the screening of an actually useful streptomycete for the fermentation process, other additional determinations are made. They are:
  - i. Screening of fermentation media through the exploitation of which the highest antibiotic yields may be obtained.
  - ii. Determination of whether the antibiotic is new.
  - iii. Determination of the number of antibiotics accumulated in the culture broth is made.
  - iv. Effect of different bioparameters on the growth of streptomycete culture, fermentation process and accumulation of antibiotic.
  - v. Solubility of antibiotic in various organic solvents and adsorbtion of antibiotic by adsorbent materials.
  - vi. Toxicity tests are conducted on mice or other laboratory animals. An antibiotic is also tested for the adverse effects if any, on man, animal or plant.
  - vii. The streptomycete culture is characterized and is classified upto species.
  - viii. Further studies on a selected streptomycete culture for mutation and strain improvement are carried out.
- Tests are designed and conducted in such a way that production streptomycete strains are obtained with least expenses.
- Similar screening and analytical techniques could be employed for the isolation of microbial isolates important in the production of other industrial chemicals.

References and Further Readings

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