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COLLEGE OF BASIC AND APPLIED SCIENCES
DEPARTMENT OF BIOLOGICAL SCIENCES

COURSE CODE: MCB 412 (3 units)
COURSE TITLE: INDUSTRIAL MICROBIOLOGY
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COURSE OUTLINE

Nature of industrial microbiology. Microorganisms of industrial importance and their products. Primary and secondary metabolites. Culture techniques and maintenance of selected cultures. Source, characteristics and improvement of industrial microorganisms. Major products of industrial microbiology. Media formulation. Fermentation system. Patents and patent law

UNIT 1: SOURCE, CHARACTERISTICS AND IMPROVEMENT OF INDUSTRIAL MICROORGANISMS

1.0 Introduction

Industrial microbiology is a broad area that deals with all forms of microbiology which have an economic effect regardless of whether it involves a fermentation product or some form of deterioration, disease or waste disposal.

A vast range of important products, many of which were formerly manufactured by chemical processes, are now most economically produced by microbial fermentation and biotransformation processes. Microorganisms have proved to be particularly **useful because** of

(i) the ease of their mass cultivation,

(ii) speed at which they grow,

(iii) use of cheap substrates that in many cases are wastes,

(iv) their ability to readily undergo genetic manipulation

(iv) and the diversity of potential products.

2.0 Objectives

By the end of this unit, you should be able to:

(a) describe how to source for a potentially industrial microorganism

(b) know the function of a culture collection and state its advantages and disadvantages.

(c) know the difference between primary and secondary screening methods.

(d) list some of the characteristics of an industrially useful microorganism.

(e) Describe “strain improvement techniques.

2.1 SOURCING FOR POTENTIALLY INDUSTRIAL MICROORGANISM

- **ISOLATION FROM THE NATURAL ENVIRONMENT**

Strategies that are adopted for the isolation of a suitable industrial microorganism from the environment can be divided into two types, ‘**shotgun**’ and **objective** approaches.

SHOTGUN APPROACH- In the shotgun approach, microbes are isolated at random from various natural sources. The best source from which to obtain a wide variety of microbes is the soil. Other sources include compost, rumen content, domestic sewage, manure, spoilt foodstuffs/feeds,, water and waste streams. Environment that have high biodiversity, extreme, and unexplored encourage the dominance of suitable microorganisms. These isolates are then screened for desirable traits.

OBJECTIVE APPROACH- involves sampling from specific sites where organisms with the desired characteristics are considered to be likely components of the natural microflora. For example, when attempting to isolate an organism that can degrade or detoxify a specific target compound, sites may be sampled that are known to be contaminated by this material. These environmental conditions may select for microorganisms able to metabolize this compound.

Once samples have been collected, an initial step is often to kill or repress the proliferation of common organisms and encourage the growth of rare ones. **Enrichment cultures** may then be performed in batch culture, or often more suitably in continuous systems. This encourages the growth of those organisms with the desired traits and increases the quantity of these target organisms, prior to isolation and screening. However, this mode of selection is suitable only for cases where the desired trait provides a competitive advantage for the organisms. Subsequent isolation as pure cultures on solid growth media involves choosing or developing the appropriate selective media and growth conditions. Once isolated as pure cultures, each must be screened for the desired property; production of a specific enzyme, inhibitory compound, etc. However, at this stage the level of activity or concentration of the target product *per se* is not of major concern, as strain development can normally be employed to vastly improve performance. Selected isolates must also be screened for other important features, such as stability and, where necessary, non-toxicity. These isolation and screening procedures are more easily applied to the search for a single microorganism. However, it is much more difficult to isolate consortia which together have the

ability/characteristic that is sought and whose composition may vary with time. Such groups can be more efficient, particularly where the ability to degrade a complex recalcitrant compound is involved.

- **CULTURE COLLECTIONS**

They serve as repositories of microbial cultures, various plasmids, cloned genes and vectors for use in genetic engineering. Microbial culture collections provide a rich source of microorganisms that are of past, present and potential future interest. There are almost 500 culture collections around the world; most of these are small, specialized collections that supply cultures or other related services only by special agreement. Others, notably national collections, publish catalogues listing the organisms held and provide extensive services for industrial and academic organizations. In the UK for example, the National Culture Collection (UKNCC) is made up of several collections. They are housed in separate institutions and tend to specialize in bacteria, yeasts, filamentous fungi or algae of either industrial or medical importance; whereas in the USA there is a main centralized collection, the American Type Culture Collection (ATCC), which holds all types of microorganisms.

FUNCTIONS- The prime functions of a culture collection are

(i) to maintain the existing collection

(ii) to continue to collect strains and

(iii) to provide pure, authenticated culture samples of each organism.

ADVANTAGE

-Use of microorganisms selected from a culture collection obviously provides significant cost savings compared with environmental isolation

-Some characterization of the microorganism will have already been performed.

DISADVANTAGE

The disadvantage is that competitors have access to the same microorganism.

2.2 SCREENING METHODS

Screening is defined as the use of highly selective procedures to allow the detection and isolation of only those microorganisms of interest from a large population. There are two methods: Primary screening and secondary screening.

Primary screening allows the detection and isolation of microorganisms that possess potentially interesting industrial applications while **Secondary screening** is usually done after primary screening to further test the capabilities of and gain information about the microorganisms.

Primary screening determines which microorganisms are able to produce a compound without providing much idea of the production or yield potential for the organisms while secondary screening allows the further sorting out of those microorganisms that have real value for industrial processes and the discarding of those lacking this potential. Secondary screening helps in predicting the approaches to be utilized in conducting further research on the microorganism and its fermentation process.

PRIMARY SCREENING METHODS

1. For detecting microrganisms producing **organic acids or amines** from various carbon substrates, a pH indicating dye such as neutral red or bromothymol blue is incorporated into a poorly buffered agar nutrient media. Only those microbes that produce considerable quantities of the acid or amines can induce changes in the color of the dye.

An alternative procedure for detecting **organic acid production** involves the incorporation of calcium carbonate in the medium so that organic acid production is indicated by a cleared zone of dissolved calcium carbonate around the colony.

Further testing e.g paper chromatography is then carried out to determine if the product is actually one of interest. Organism showing fermentation potential is immediately purified and subcultured into appropriate agar medium to be maintained as stock cultures.

2. For microbes capable of producing useful **antibiotics**, the simplest screening technique is the "crowded plate' technique. Soil or any other source of microbe is diluted to a concentration that will yield individual colonies on the surface of the agar. Colonies producing antibiotic activity are indicated by an area of agar around the colony that is free of growth of other colonies. Such a colony is subcultured to a similar medium and purified by streaking before making stock cultures. The purified culture is further tested for the microbial inhibition spectrum.

3. To screen for microorganisms capable of synthesizing **extracellular vitamins** or other metabolites, the medium at make-up must be totally lacking in the metabolite under consideration. 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 test organism must possess a definite growth

requirement for the particular metabolite, so that the production of this compound will be indicated by zones of growth of the test organism adjacent to colonies that have produced the metabolite.

4. To screen a microbial source in order to find microbial source capable of utilizing a specific carbon or nitrogen nutrient for growth and biosynthesis, the plating medium is made up so as to contain the particular nutrient as its only source. Dilutions of soil are applied to the plates and the colonies appearing after incubation are assumed to possess the desired attribute. Further testing is required to ensure that the growth is actually attributed to the nutrient source.

SECONDARY SCREENING

All primary screening techniques is usually followed by a secondary screening technique to further test the capabilities and gain information about these organisms. Secondary screening is conducted on agar plates, in flasks or in small fermenters containing liquid media. Use of liquid media provides a better picture of the actual yield potentials among various isolates.

A broad range of information that can be provided from secondary screening include

(i) Secondary screening can be qualitative or quantitative in its approach. Using antibiotics as an example, qualitative tells us the range of microorganisms sensitive to the antibiotic while quantitative indicates the yield of the antibiotic when the microorganisms is grown in various differing media.

(ii) secondary screening should determine what type of microbes are involved and whether they can be classified to families or genera. The organism should be classified as to species by the time a patent application is filed.

(iii) secondary screening should determine whether the microbes are actually producing new chemical compounds that are useful or whether a more economic fermentation process is possible. To discover whether a product is a newly discovered compound, chromatographic procedures are used to compare the products with known compounds.

(iv) should detect real differences in product yield potential among various isolates. The organisms are grown for various periods of time and on various media so that quantitative assay may be performed.

(v) should reveal whether there are pH, aeration or other critical requirements for growth or product formation.

(vi) should reveal the physical and chemical properties of the product, also the toxicity of the product.

2.3 CHARACTERISTICS OF INDUSTRIAL MICRORGANISM

Irrespective of the origins of an industrial microorganism, it should ideally exhibit:

1 genetic stability;

2 efficient production of the target product, whose route of biosynthesis should preferably be well characterized;

3 limited or no need for vitamins and additional growth factors;

4 utilization of a wide range of low-cost and readily available carbon sources;

5 amenability to genetic manipulation;

6 safety, non-pathogenicity and should not produce toxic agents, unless this is the target product;

7 ready harvesting from the fermentation;

8 ready breakage, if the target product is intracellular;

9 production of limited byproducts to ease subsequent purification problems.

2.4 STRAIN IMPROVEMENT.

Newly isolated microorganisms often produce a secondary metabolite of interest in low concentrations. Once a compound with interesting activity is found from a newly isolated microbe, it is desirable to produce more of this compound for further testing and characterization. This is achieved most simply by

(i) growing larger volumes of culture

(ii) altering the composition of growth medium and culture conditions

(iii) by generating altered genotype to obtain improvement in the yield or in other characteristics since the maximum potential of the strain to produce a metabolite is determined by the genome.

Strain improvement can be defined as the process of generating altered genotypes followed by the examination of the resulting strains for an improved titre of products or any desired characteristics.

GENERATION OF ALTERED GENOTYPE- this can arise as a consequence of the following:

1. **NATURAL MUTATION -** The frequency of spontaneous mutations is normally so low that the screening of large number of strains which would be necessary is not feasible. To increase the frequency of mutation, it is necessary to resolve to the use of mutagenic agents.

2. USE OF MUTAGENIC AGENTS - The use of mutagens frequently generate mutation not found amongst spontaneous mutations. A variety of mutagens may be employed including radiation (UV, Gamma and X-irradiation) and chemical mutagens such as ethyl methane sulphonate (EMS), nitro-nitroso-methylguanidine and the mustards. The type of mutation produced will be dependent on the type of DNA damage caused by the mutagen and the action of the cellular DNA repair pathways on the damage. Most induced mutations are deleterious to the yield of the desired product, however, a minority are more productive than the parent strain.

3. RECOMBINATION – Natural methods of DNA transfer between cells which may lead to recombination include transformation, conjugation and transduction (in cases of bacteria) and heterokaryosis and sexual cycle in fungi. Although these may all find some application in strain improvement, the most widely used method of genetic transfer are now those which are unlikely to occur in nature e.g parasexual breeding, protoplast fusion and genetic engineering. Protoplast fusion is largely used with yeast and molds, most of which are asexual or single mating types. The cells are grown in an isotonic solution while treating them with enzymes.

3.0 Tutored-Marked Assignment

1. Describe how you would source for an organism capable of producing a useful antibiotic.
2. State the difference between the primary and secondary screening method.
3. State five characteristics of an industrially useful microrganism.
4. List the prime functions of a culture collection.
5. List and explain the methods for stain improvement.

4.0 References/Further Readings

- Casida, L.E.JR .(2007). Industrial Microbiology. New Age International Limited Publisher
- Waites, M.J., Morgan, N.L, Rockey, J.S and Higton, G. (2001). Industrial Microbiology: An Introduction. Blackwell Science Ltd.