SHREE H. N. SHUKLACOLLEGE OF SCIENCE

(AFFILIATED TO SAURASHTRA UNIVERSITY)

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F.Y. B.Sc. (Sem. I) (CBCS)

MICROBIOLOGY

MB-101- FUNDAMENTALS OF MICROBIOLOGY

Unit 4

CULTIVATION OF BACTERA

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CONTENT

- Nutritional requirements and types of Bacteria,
- Chemical and Physical requirement of Growth – Bacteriological Media & their Types, Air, pH & Temperature, Cultivation of Anaerobes
- Natural Microbial Population (Mixed Cultures), Selective methods to obtain Pure Cultures
- Isolation and Preservation of pure cultures
- Cultural Characteristics

TITLE EXPLANATION

- In this unit we are going to discuss about cultivation of bacteria.
- We can study about nutritional requirement and nutritional types pf bacteria.
- Study about chemical and physical requirement of growth.
- Stucy about natural microbial populaiton and how to obtain pure culture from mixed culture or natural populaiton.
- Learnirng about isolation and preservation techniques of pure cultures.

LEARNING OUTCOME

- Microbial cultures are **used to determine the type of organism**, its abundance in the sample being tested, or both. It is one of the primary diagnostic methods of microbiology and used as a tool to determine the cause of infectious disease by letting the agent multiply in a predetermined medium.
- Study of cultural characteristics of microorganism is done with a purpose to distinguish different microorganisms into various taxonomic groups. So the objective of this experiment is **to understand different characteristics of microorganisms and to classify them into various groups basing on their characteristics**.

For study purpose

Nutritional requirements and types of Bacteria

Bacteria are prokaryotic organisms that do not contain chlorophyll. They are unicellular and do not show true branching. They differ from eukaryotes in not having a nuclear membrane, a nucleolus, and cell organelles like mitochondria, golgi apparatus and endoplasmic reticulum. They have a single circular chromosome. This chapter will deal with the growth and multiplication of bacteria and their requirements for the same. It will deal with the energy requirements and their ability to synthesise essential metabolites.

Bacterial nutrition

- The bacterial cell has the same general chemical pattern as the cells of other organisms. The bacterial cell contains water (80% of total weight), proteins, polysaccharides, lipids, nucleic acids, mucopeptides and low molecular weight compounds.
- For growth and nutrition of bacteria, the minimum nutritional requirements are water, a source of carbon, a source of nitrogen and some inorganic salts. Water is the vehicle of entry of all nutrients into the cell and for the elimination of waste products. Bacteria can be classified nutritionally based on their energy requirements and on their ability to synthesise essential metabolites. Bacteria which derive energy from sunlight are called phototrophs.
- Those that obtain energy from chemical reactions are called chemotrophs. Bacteria that can synthesise all their organic compounds are called autotrophs. They are able to use atmospheric carbon dioxide and nitrogen. They are capable of independent existence in water and soil. They are of no medical importance. Some bacteria are unable to synthesise their own metabolites. They depend on preformed organic compounds.
- They are called heterotrophs. These bacteria are unable to grow with carbon dioxide as the sole source of carbon. Their nutritional requirements vary widely. Some may require only a single organic substance like glucose. Others may need a large number of different compounds like amino acids, nucleotides, lipids, carbohydrates and coenzymes.
- Bacteria require a supply of inorganic salts. They require anions like phosphate and sulphate anions and cations like sodium, potassium, magnesium, iron and calcium. Some ions like cobalt may be required in trace amounts. Some bacteria require certain organic compounds in minute quantities. These are called growth factors or bacterial vitamins.
- Growth factors are called essential when growth does not occur in their absence. Accessory growth factors are those which enhance growth without being absolutely necessary for it. In many cases, bacterial vitamins are same as vitamins necessary for nutrition of mammals, for example, B group vitamins – thiamine, riboflavin, pyridoxine, nicotinic acid, folic acid and vitamin B12.

Gaseous Requirements

- Depending on the influence of oxygen on growth and survival, bacteria are divided into aerobes and anaerobes. Aerobic bacteria require oxygen for growth. They may be obligate aerobes or facultative anaerobes. Obligate aerobes grow only in the presence of oxygen, for eg. Cholera bacillus.
- Facultative anaerobes are ordinarily aerobic but can grow in the absence of oxygen, though less abundantly. Most bacteria of medical importance are facultative anaerobes. Anaerobic bacteria, such as clostridia grow in the absence of oxygen. Obligate anaerobes may even die on exposure to oxygen. Microaerophilic bacteria are those that grow best in the presence of low oxygen tension.
- In case of aerobes, atmospheric oxygen is the final electron acceptor in the process of respiration (aerobic respiration). In this case, the carbon and energy source may be completely oxidised to carbon dioxide and water. Energy is provided by the production of energy-rich phosphate bonds and the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). This process is called oxidative phosphorylation.
- Anaerobic bacteria use compounds like nitrates or sulphates instead of oxygen as final electron acceptors in the process of respiration (anaerobic respiration). A more common process used by these bacteria in anaerobic metabolism is fermentation. It is defined as the process by which complex organic compounds, such as glucose, are broken down by the action of enzymes into simpler compounds without the use of oxygen.
- This process leads to the formation of several organic end products such as organic acids and alcohols, as well as of gas (carbon dioxide and hydrogen). For example, Escherichia coli ferments glucose with the production of acid and gas. It also ferments lactose. During the process of fermentation, energy-rich phosphate bonds are produced by the introduction of organic phosphate into intermediate metabolites.
- This process is known as substrate-level phosphorylation. The energy-rich phosphate groups so formed are used for conversion of ADP to ATP. All bacteria require some amounts of carbon dioxide for growth. This is obtained from the atmosphere or from the cellular metabolism of the bacterial cell. Some bacteria like Brucella abortus require much higher levels of carbon dioxide (5- 10%) for growth. They are called capnophilic.

Temperature Requirements

- Bacteria vary in their requirement of temperature for growth. For each species, there is a “temperature range”, and growth does not occur above the maximum or below the minimum of this range.
- The temperature at which growth occurs best is known as the “optimum temperature”. In the case of most pathogenic bacteria, the optimum temperature is 37ºC. Bacteria which

grow best at temperatures of 25-40ºC are called mesophilic, for example Escherichia coli. Psychrophilic bacteria are those that grow best at temperatures below 20ºC.

- They are soil and water saprophytes and may cause spoilage of refrigerated food. Thermophilic bacteria are those which grow best at high temperatures, 55-80ºC.
- They may cause spoilage of underprocessed canned food. Some thermophiles, for example Geobacillus stearothermophilus, form spores that are highly thermoresistant.

Other physiological Requirements

- Moisture and drying, hydrogen ion concentration, light, osmotic effect and mechanical and sonic stress may also influence the growth and multiplication of bacteria.

CHEMICAL AND PHYSICAL REQUIREMENT OF GROWTH

1) BACTERIOLOGICAL MEDIA: a culture is a solid or liquid preparation used to grow, transport and store microorganisms.

Components of media: the study of microorganisms requires being able to grow them in the laboratory. Bacteria are grown in culture media, which provides the nutrients, necessary for the organism of interest.

Protein/peptides/amino acids: bacteria require nitrogen to make proteins and nucleic acid. Only a few genera of bacteria can use free molecular nitrogen from the air.

Others required fixed nitrogen in the organic form. Some can use nitrate or nitrite salts, but most require animo acids, peptides, peptones or proteins.

2. energy : the most common substance added to culture media as a source of energy to increase the rate of growth of organisms is glucose. Other carbohydrates may be used as required. Carbohydrate added to media at 5-10 grams per litre are usually present as biochemical substrates to detect the production of specific enzymes in the identification of organisms. It is usual to add pH indicator to such formulaitons.

Essential metals and minerals: the inorganic essential components of culture media are many and can be divide on a semi-quantatiave basis. Typical macro-components: Na, K, Cl, P, S, Ca,Mg,Fe

Typical micro components: Zn, Mn, Br, B, Cu,Co, Mo,Sr

Buffering agents: phosphate, acetate, citrate, zwitterion compounds and specific amino-acids are example of buffering agents that may be added to culture media.

Indicator for pH change: phenol red, bromo-cresol purple, fuchsin are used in media for indicator.

Selective agents: chemicals, antimicrobial agents.

The selective agents are chosen and added at specific concentration to supress the growth of unwanted organisms in a polymicrobial sample. It is essential to have established that the selective agents, at the appropriate concentration, will allow uninhibited growth of desired organisms.

Common chemical selective agents are: bile salts, dye stuffs, selenite, tetrathionate, tellurite and azide.

Gelling agent: usually agar

Although gelatin is still used for a few specific media and carrageenans, alginates, silica gel and polyacrylamides are sometimes used as gelling agents.

Agar is obtained from agarophyte sea weeds mainly *Gelidium, Garcilariand, Pterocladia* . It is extracted as an aquous solution at greater than 100degree C, decolorised, filtered, dried and milled to a power.

BACTERIOLOGICAL MEDIA AND THEIR TYPES

there are different way to classify the media.

CONSISTENCY:

Liquid media: In liquid medium, bacteria grow producing turbidity/ surface pellicle (Vibrio & Bacillus)/ granular deposits (Streptococci). Culturing bacteria in liquid media has some drawbacks. Properties of bacteria are not visible in liquid media and presence of more than one type of bacteria cannot be detected.

Solid media

Any liquid medium can be rendered solid by the addition of certain solidifying agents. Agar agar (simply called agar) is the most commonly used solidifying agent. It is an unbranched polysaccharide obtained from the cell membranes of some species of red algae such as the genera Gelidium. Agar is composed of two long-chain polysaccharides (70% agarose and 30% agarapectin). It melts at 95°C and solidifies at 42oC, doesn't contribute any nutritive property, it is not hydrolysed by most bacteria and is usually free from growth promoting or growth retarding substances. Agar is available as powders.

Semi-solid media

Reducing the amount of agar to 0.2-0.5% renders a medium semi-solid. Such media are fairly soft and are useful in demonstrating bacterial motility (U-tube and Cragie's tube). Certain transport media such as Stuart's and Amies media are semi-solid in consistency. Hugh & Leifson's oxidation fermentation test medium as well as mannitol motility medium are also semi-solid.

nutrient media: is a specific chemical formulations that contain all the nutrients and minerals that a many microorganisms needs for normal growth. It is called defined when the specific nutrients and their amounts are known. An undefined media is one where the exact composition is not known.

Selective media: is a type of media that favors the growth of a specific microorganisms over others. In fact others may be inhibited by media components.

Differential media: permit the recognition of specific microorganisms. Usually by taking advantage of some biochemical reaction that might produce a specific change in the media.

Defined/synthetic media: media whose composition are chemically defined. contain highly pure organic and inorganic compounds that very little from one source from another. Have a molecular content specified by means of an exact formula. All the ingredients of a culture medium are known, both qualitatively and quantitatively. Great value in studying the nutritional requirements of microorganisms or in studying a great variety of their metabolic activities.

Complex/non-synthetic media: contain at least one ingredient that is not chemically definable. Not a simple, pure compound and not representable by an exact chemical formula exact chemical composition is not known, and such a medium is often prepared from very complex materials. Contains most of the organic compounds-sugars, amino acids, and nucleotides necessary for growth contains nutrients released by the partial digestion of yeast, beef, soy or proteins.

Enrichment media: media used to enhance the growth of the desired organisms in a mixed population; similar to selective media to a detectable level without stimulating the rest of the bacterial population.

Selective media: contain one or more agents that inhibits the growth of a certain microbe(A,B,C) or microbes but not others(D), and thereby encourages or selects microbe D and allows it to grow. Used in primary isolation of a specific type of microorganisms from sample containing a highly mixed population. Grow media that contains substance that inhabit the growth of unwanted organisms but permit the growth of the desired organisms- uses certain dyes, high salt concentration, pH or antibiotics.

Examples of selective media include:

→ **mannitol salt agar(**selects against non-skin flora)

→ **Macconkey agar(**selects against gram-positive)

→ **Eosin methyle blue agar(**selects against gram positive)

→ **phenylethyl alcohol agar(**selects against gram negative)

Eosin, methylene blue, crystal violet dyes (inhibit the growth of gram positive bacteria without affecting gram negative). it supports the growth of desired organisms while inhibiting the growth of many or most of the unwanted ones-either by purposely adding one or more selective agents which “poison” certain types of organisms or by including or deleting certain nutrients such that the desired organisms and few others are able to grow.

Differential medium: can grow several types of microorganisms but it is designed to highlight differences among these microorganisms. Allow the growth of more than one microorganisms. Allow the growth of more than one microorganisms of interest but with morphologically distinguishable colonies. Contains a combination of nutrient and pH indicators to visually differentiate bacteria that grow on or In it. frequently a solid medium on which colonies of a particular species will have a distinctive color or cause a change in the color of the medium.

Examples of differential media include:

→**mannitol salt agar (**mannitol fermentation)

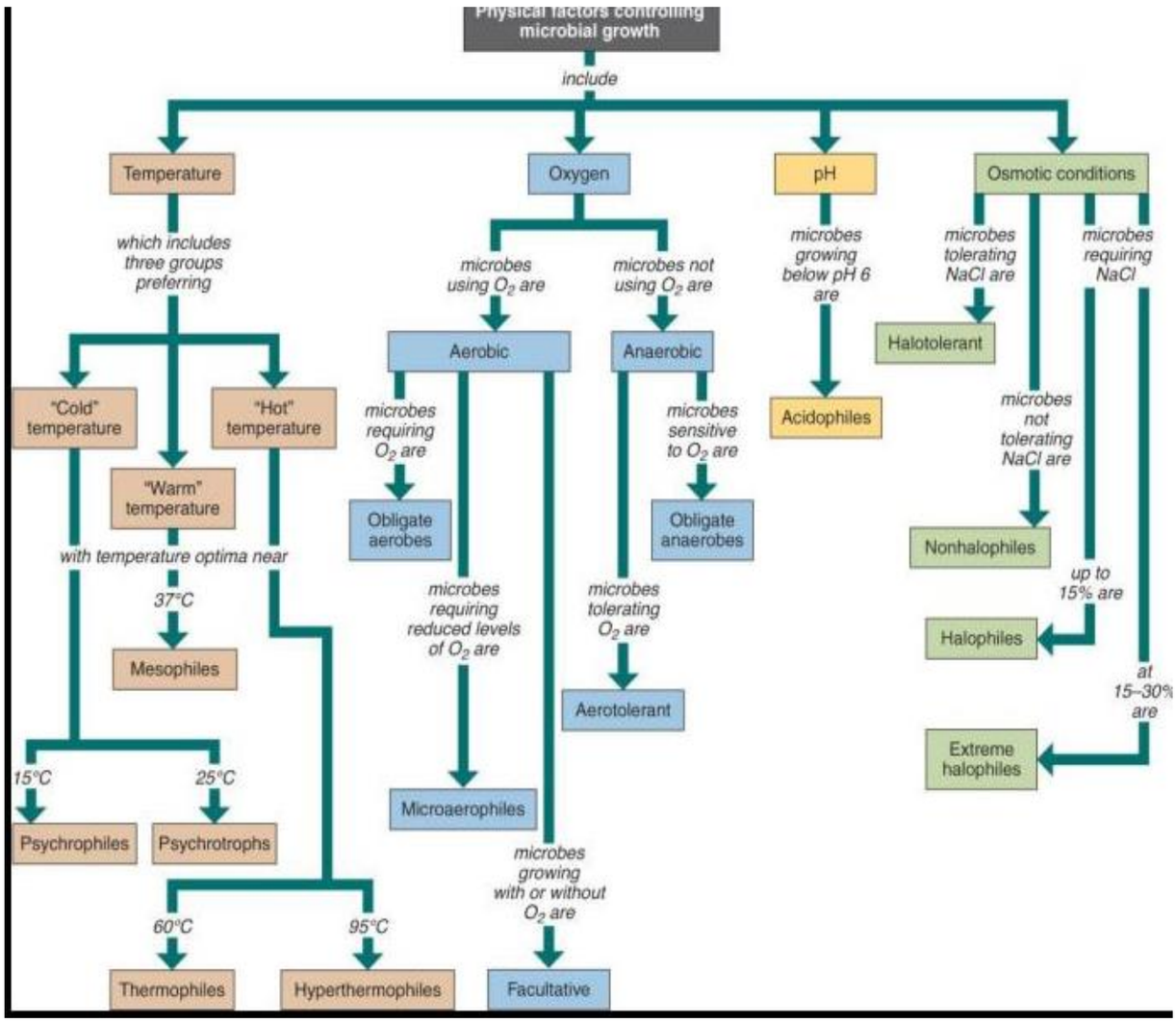
→**blood agar**(various kinds of hemolysis)

→**Macconkey agar(**lactose fermentation)

→ **Eosin methylene blue agar(** various kinds of differentiation)

allows two or more different organisms to grow, but it contains dyes and/or other components upon which different organisms act in various ways to produce a variety of end products or effects, often detected by variations in color. These differences are often very apparent among colonies of a mixed culture growing in a petri dish. Pure cultures, growing tubes of the same differential medium, may also be characterized from one another according to a particular biochemical characteristic.

TYPES OF BACTERIA



ISOLATION OF MICROBES

- **Culture**

  The organism growing on the media plate is called as culture.

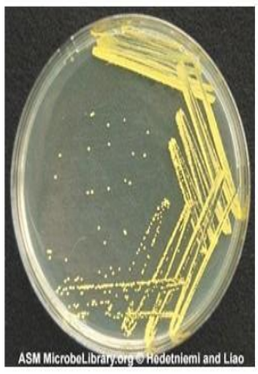
- **Colony**

The number of cells of any organism living together.

Culture Types of

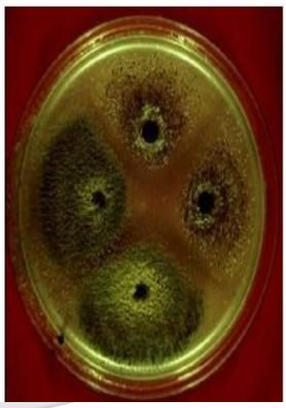
1-Pure Culture

Only one type of microorganism growing on the media plate



2-Contaminated culture

More than one type of organism growing on the media plate



Purification of microorganism

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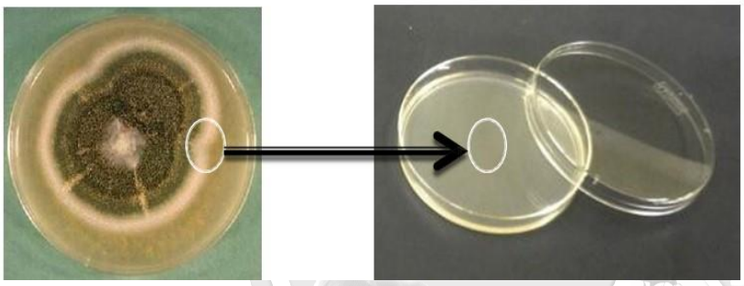
Purification of Fungi:

1. Use a cork borer or pasture pipet.
2. Flame cork borer using alcohol 70% and allow to cool.
3. Cut few discs from the edge of an actively growing fungal colony.
4. Inoculate it (surface facing down) on the center another media plate with the help of flamed forceps
5. Incubate it for 3-5 days
6. Pure culture of the organism will grow.

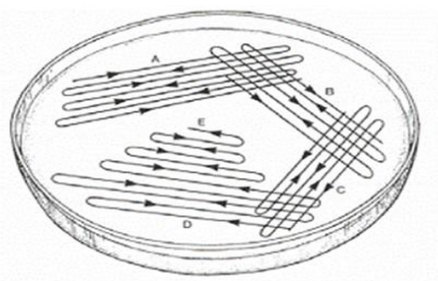


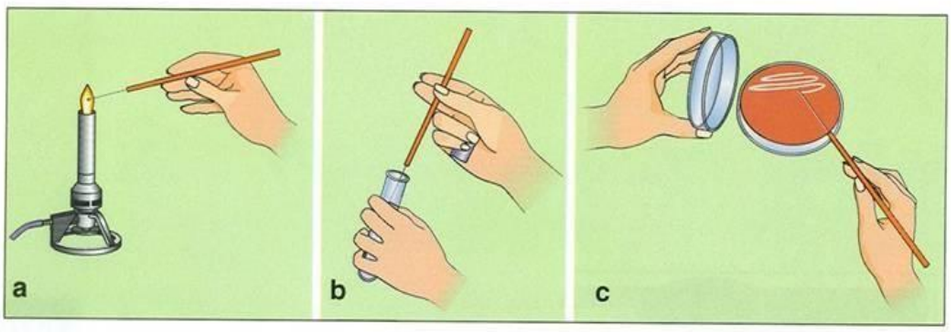
Purification of Bacteria :

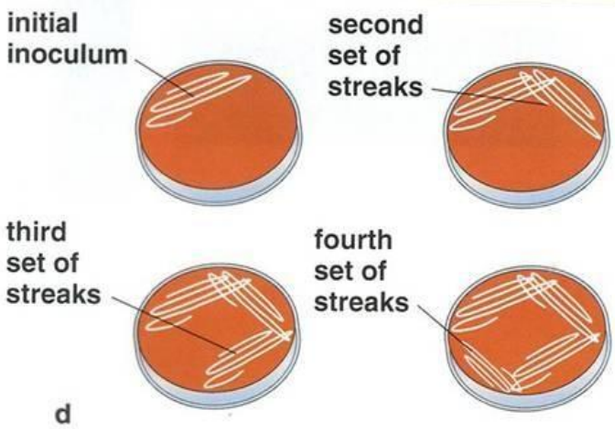
Streak plate Method

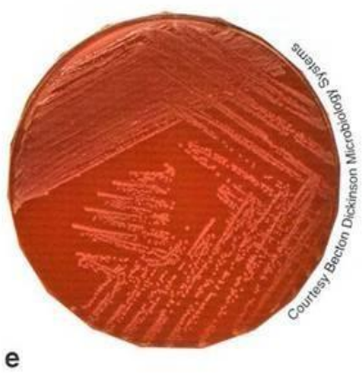
- Streak inoculum onto one portion of the plate
- Sterilize the loop
- Streak through the first inoculum and spread into second section
- Repeat several times
- Incubate it for 24 h. ,

a single colony will be observed .









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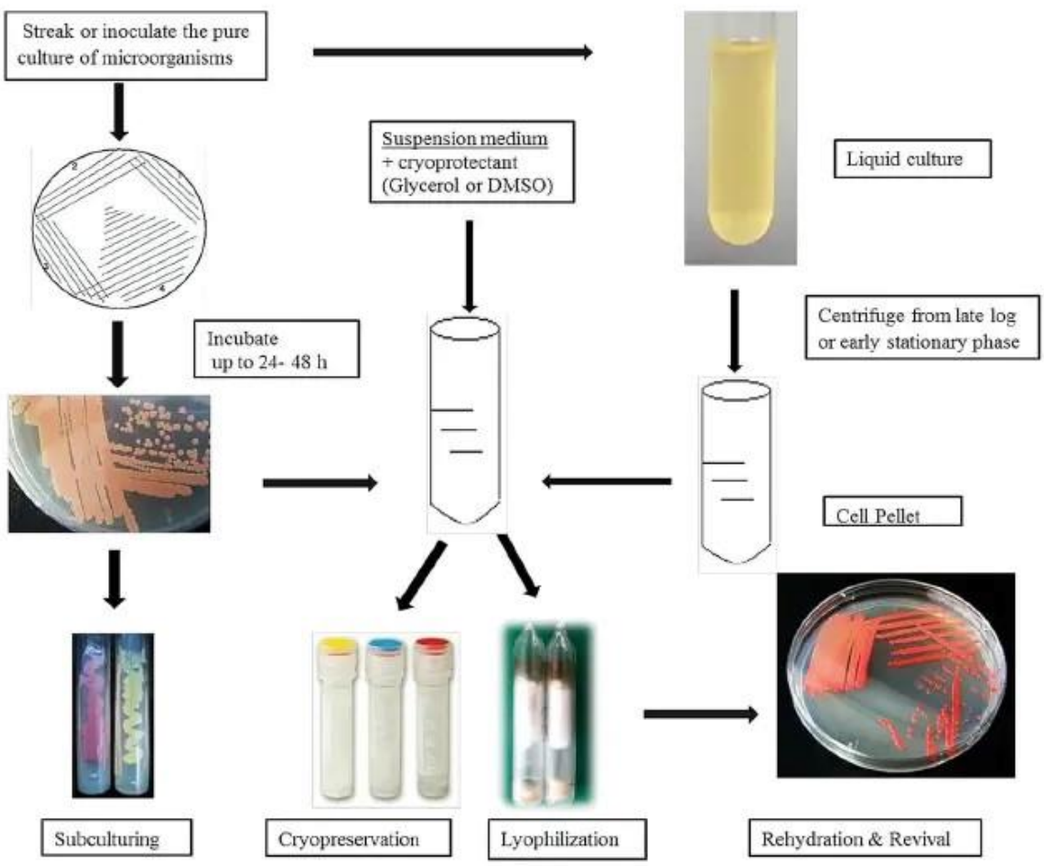

CULTURAL CHARACTERISTICS

- **Form**

What is the basic shape of the colony? For example, circular, filamentous, etc.

- **Margin**

What is the magnified shape of the edge of the colony?

- **Elevation**

What is the cross sectional shape of the colony? Turn the Petri dish on end.

- **Surface**

How does the surface of the colony appear? For example, smooth, rough, dull , wrinkled etc.

- **Pigmentation**

For example, white, red, purple, etc.