Physiology of Bacteria

Microbial Metabolism

- The primary function of all living cells is to grow and reproduce
- Growth & reproduction rely on the outcome of chemical reactions in the cells
- The sum of all cellular chemical reactions is referred to as **metabolism**

Microbial Metabolism

- The metabolic process that involves the **degradation** of chemical components is called **catabolism**

- The synthesis of chemical components is called **anabolism** or **biosynthesis**

- Most metabolic processes in the cell would take forever if it were not for **enzymes**
- **Enzymes** are proteins that have molecular weights ranging from 600 to 12 000
  - **Their function is to speed up the various chemical reactions that occur in the cell**
  - **Molecules that speed up chemical reactions are called catalysts**
  - **Enzymes often cannot function alone and require additional molecules, called cofactors, to enhance activity**

Classification of enzymes

- **Oxidoreductases** are involved in electron (hydrogen) transfer reactions
- **Transferases** transfer specific groups such as aldehydes or phosphates from one substrate to another
- **Hydrolyses** add water across chemical bonds to be cleaved or hydrolyzed

Classification of enzymes

- **Lyases** remove chemical groups from substrates, forming double bonds, or add chemical groups to double bonds
- **Isomerases** rearrange certain compounds to produce molecules having the same groups of atoms, but in different arrangements
- **Ligases** produce bonds accompanied by the cleavage of ATP

Classification of enzymes

- Enzymes synthesized by the cell remain within the cell to carry out specific reactions and are called **endoenzymes**

- Enzymes relased from the cell into the surrounding environment and are called **exoenzymes**

Classification of enzymes

- **Pathogenicity enzymes** – are enzymes that damage cells and tissues
  - **Coagulase – enables the organisms to clot plasma to form a sticky coat of fibrin around themselves for protection from phagocytes and other body defense machanisms (Staphylococcus)**
  - **Kinases – reffered to as fibrinolysin, kinase has opposite effect of coagulase. Streptokinase, for example, lyses fibrin clots, thus enabling streptococci to invade and spread throughout the body**

Classification of enzymes

- **Hyaluronidase** – enables pathogens to spread through connective tissue by breaking down hyaluronic acid, the "cement" that holds tissue cells together (Staphylococcus, Streptococcus and Clostridium)
- **Collagenase** – This enzyme breaks down collagen, the supportive protein founding tendons, cartilage and bones. Cl. perfringens a major cause of gas gangrene, spreads deeply within the body by secreting both collagenase and hyaluronidase

Classification of enzymes

- **Hemolysin** – enzyme that cause damage to the host's red blood cells. In the laboratory, hemolysis of the red blood cells in the blood agar is useful for identifying types of Staphylococcus and Streptococcus
- **Lecithinase** – one of the toxins produced by Staphylococcus aureus, which breaks down phospholipids collectively referred to as lecithin
- **Leukocidin** – enzyme secreted some Staphylococcus aureus causes destruction

Classification of enzymes

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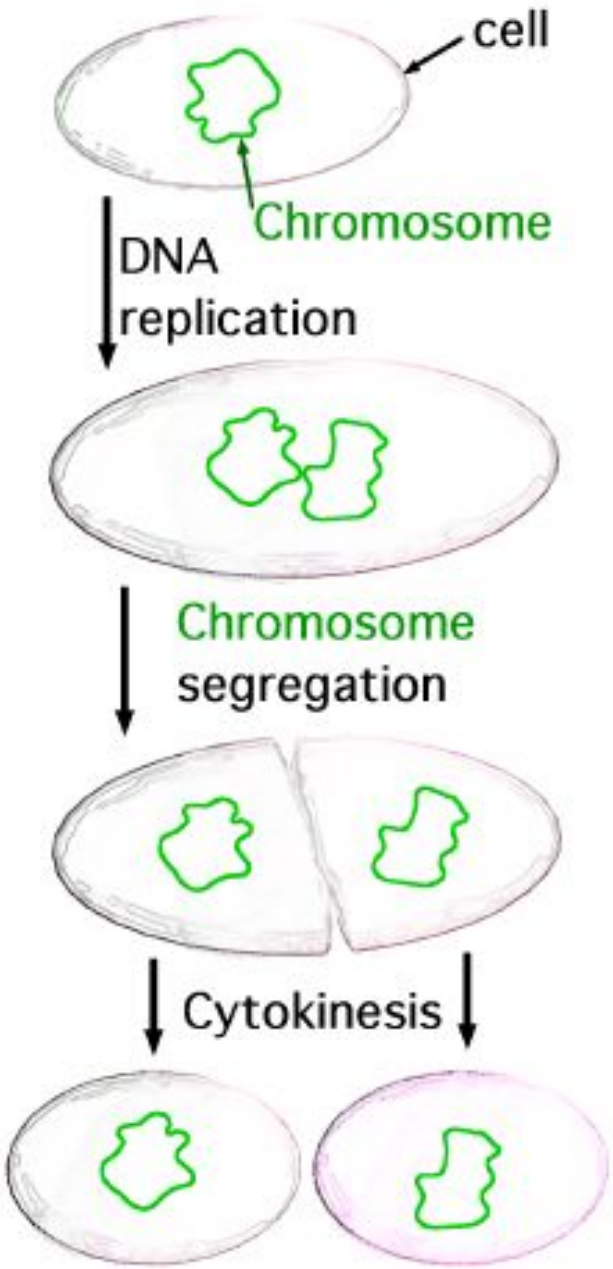
Growth & Multiplication of Bacteria

- Bacteria divide by binary fission
- Bacterial cell divides to form two daughter cells
- Nuclear division precedes cell division & in a growing population many cells carrying two nuclear bodies can be seen


cell
Chromosome
DNA
replication
Chromosome
segregation
Cytokinesis

- The interval of time between two cell division, or the time required for a bacterium to give rise to two daughter cells under optimum conditions, is known as the generation time or population doubling time

Growth & Multiplication of Bacteria

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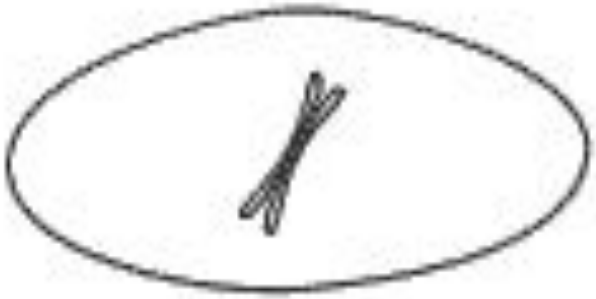

- In many medically important bacteria, the generation time is about 20 minutes
- Some bacteria are slow-growing
  - **Tubercle bacilli the generation time is about 20 hours**
  - **Lepra bacilli about 20 days**

- Bacteria reproduce so rapidly & by geometric progression, a single bacterial cell can theoretically give rise to 1021 progeny in 24 hours, with a mass of approximately 4,000 tones!

- When bacteria are grown in a vessel of liquid medium (batch culture), multiplication is arrested after a few cell divisions due to depletion of nutrients or accumulation of toxic products
- When pathogenic bacteria multiply in host tissues, the situation may be intermediate between a batch culture & a continuous culture
- Bacteria growing on solid media form colonies
- Each colony represents a clone of cells derived from a single parent cell
- In liquid media, growth is diffuse

Bacterial cell Growth Curve

- **A- Lag phase**
  - **Immediately following the seeding of a culture medium**
  - **This initial period is the time required for adaptation to the new environment**
  - **There is no increase in numbers, though there may be an increase in the size of the cells**
- **B- Log (logarithmic) or exponential phase**
  - **The cells start dividing & their numbers increase exponentially or by geometric progression**

Bacterial cell Growth Curve

- **C- Stationary phase**
  - **After a period of exponential growth, cell division stops due to depletion of nutrients & accumulation of toxic products**
  - **The viable count remains stationary as an equilibrium exists between the dying cells and the newly formed cells**
- **D- Phase of Decline**
  - **Population decreases due to cell death**

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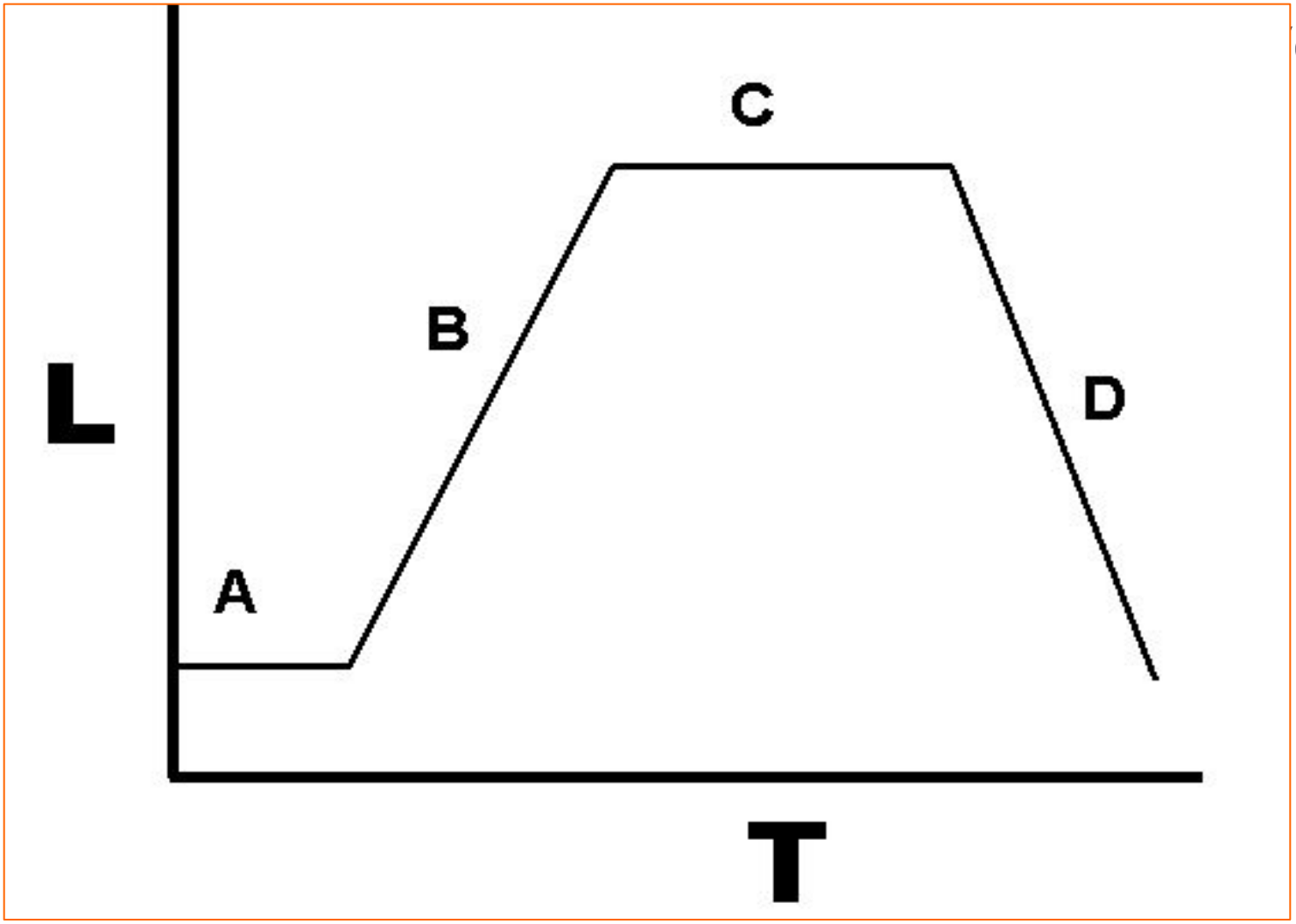


Nutritional requirements

- Microorganisms also depend on an available source of chemical nutrients. Microorganisms are often grouped according to their energy source and their source of carbon.
- a. **Energy source**
  - 1. **Phototrophs** use radiant energy (light) as their primary energy source.
  - 2. **Chemotrophs** use the oxidation and reduction of chemical compounds as their primary energy source.
- b. **Carbon source**
- Based on their source of carbon bacteria can be classified as autotrophs or heterotrophs.
  - 1. **Autotrophs**: require only carbon dioxide as a carbon source. An autotroph can synthesize organic molecules from inorganic nutrients.
  - 2. **Heterotrophs**: require organic forms of carbon. A Heterotroph cannot synthesize organic molecules from inorganic nutrients.

Nutritional types in bacterial metabolism

Nutritional type	Source of energy	Source of carbon	Examples
Phototrophs	Sunlight	Organic compounds (photoheterotrophs) or carbon fixation (photoautotrophs)	Cyanobacteria, Green sulfur bacteria, Chloroflexi, or Purple bacteria
Lithotrophs	Inorganic compounds	Organic compounds (lithoheterotrophs) or carbon fixation (lithoautotrophs)	Thermodesulfobacteria, *Hydrogenophilaceae*, or Nitrospirae
Organotrophs	Organic compounds	Organic compounds (chemoheterotrophs) or carbon fixation (chemoautotrophs)	*Bacillus*, *Clostridium* or *Enterobacteriaceae*

All organisms in nature can be placed into one of four separate groups: photoautotrophs, photoheterotrophs, chemoautotrophs, and chemoheterotrophs.

- 1. **Photoautotrophs** use **light** as an energy source and **carbon dioxide** as their main carbon source. They include photosynthetic bacteria (green sulfur bacteria, purple sulfur bacteria, and cyanobacteria), algae, and green plants. Photoautotrophs transform carbon dioxide and water into carbohydrates and oxygen gas through **photosynthesis**.

- 2. **Photoheterotrophs** use **light** as an energy source but cannot convert carbon dioxide into energy.. They include the green nonsulfur bacteria and the purple nonsulfur bacteria.

- 3. **Chemolithoautotrophs** use **inorganic compounds** such as hydrogen sulfide, sulfur, ammonia, nitrites, hydrogen gas, or iron as an energy source and **carbon dioxide** as their main carbon source.

- 4. **Chemooganoheterotrophs** use **organic compounds** as both an energy source and a carbon source. **Saprophytes** live on dead organic matter while **parasites** get their nutrients from a living host. Most bacteria, & all protozoans, fungi, and animals are chemoorganoheterotrophs.

Nutritional requirements

- C. **Minerals**
- 1. **sulfur** - Sulfur is needed to synthesizes sulfur-containing amino acids and certain vitamins.
- 2. **phosphorus** - Phosphorus is needed to synthesize phospholipids *(def)*, DNA, RNA, and ATP *(def)*. Phosphate ions are the primary source of phosphorus.
- 3. **potassium, magnesium, and calcium** - These are required for certain enzymes to function as well as additional functions.
- 4. **iron** - Iron is a part of certain enzymes.
- 5. **trace elements** - Trace elements are elements required in very minute amounts, and like potassium, magnesium, calcium, and iron, they usually function as **cofactors** *(def)* in enzyme reactions. They include sodium, zinc, copper,molybdenum, manganese, and cobalt ions. Cofactors usually function as electron donors or electron acceptors during enzyme reactions.

Nutritional requirements

- D. **Water**

- E. **Growth factors**
  Growth factors are organic compounds such as amino acids *(def)*, purines *(def)*, pyrimidines *(def)*, and vitamins *(def)* that a cell must have for growth but cannot synthesize itself. Organisms having complex nutritional requirements and needing many growth factors are said to be **fastidious**.

Oxygen Requirements

- Depending on the influence of oxygen on growth and viability, bacteria are divided into **aerobes & anaerobes**
- Aerobic bacteria require oxygen for growth

Aerobic bacteria

↓

obligate aerobes

(Vibrio cholerae)

Oxygen Requirements

- Anaerobic bacteria grow only in absence of oxygen

Anaerobic bacteria

obligate anaerobe (clostridia)

facultative anaerobes (most of medically important bacteria)

Oxygen requirements can be classified

- **Obligate aerobes — which** can grow only in the presence of oxygen (e.g., P. aeruginosa)
- **Obligate anaerobes** are organisms that grow **only** in the absence of oxygen and, in fact, are often inhibited or killed by its presence. They obtain their energy through anaerobic respiration or fermentation. (e.g., Clostridium botulinum Clostridium tetani, etc.)
- **Facultative anaerobes** which are ordinary aerobes but can also grow without oxygen (e.g., E. coli). Most of the pathogenic bacteria are facultative aerobes.

Oxygen requirements can be classified

- **Microaerophiles** are organisms that require a low concentration of oxygen (2% to 10%) for growth, but higher concentrations are inhibitory. They obtain their energy through aerobic respiration. (e.g., Campylobacter jejuni).
- **Aerotolerant anaerobes** like obligate anaerobes, cannot use oxygen to transform energy but can grow in its presence. They obtain energy only by fermentation and are known as obligate fermenters.

Physical requirements

- **Temperature**
  - 1. **Psychrophiles** are cold-loving bacteria. Their optimum growth temperature is between -5C and 15C. They are usually found in the Arctic and Antarctic regions and in streams fed by glaciers.
  - 2. **Mesophiles** are bacteria that grow best at moderate temperatures. Their optimum growth temperature is between 25C and 45C. Most bacteria are mesophilic and include common soil bacteria and bacteria that live in and on the body.

- **pH**
- Microorganisms can be placed in one of the following groups based on their optimum pH requirements:
- 1. Neutrophiles grow best at a pH range of 5 to 8.
- 2. Acidophiles grow best at a pH below 5.5.
- 3. Allaliphiles grow best at a pH above 8.5.

Culture Media

- A **growth medium** or **culture medium** is a substance in which microorganisms or cells can grow
- There are two major types of growth media: those used for cell culture, which use specific cell types derived from plants or animals, and microbiological culture, which are used for growing microorganisms, such as bacteria or yeast

Types of Growth Media

- The most common growth media for microorganisms are *nutrient broths* (liquid nutrient medium) or *Lysogeny broth* (LB medium). Bacteria grown in liquid cultures often form colloidal suspensions.

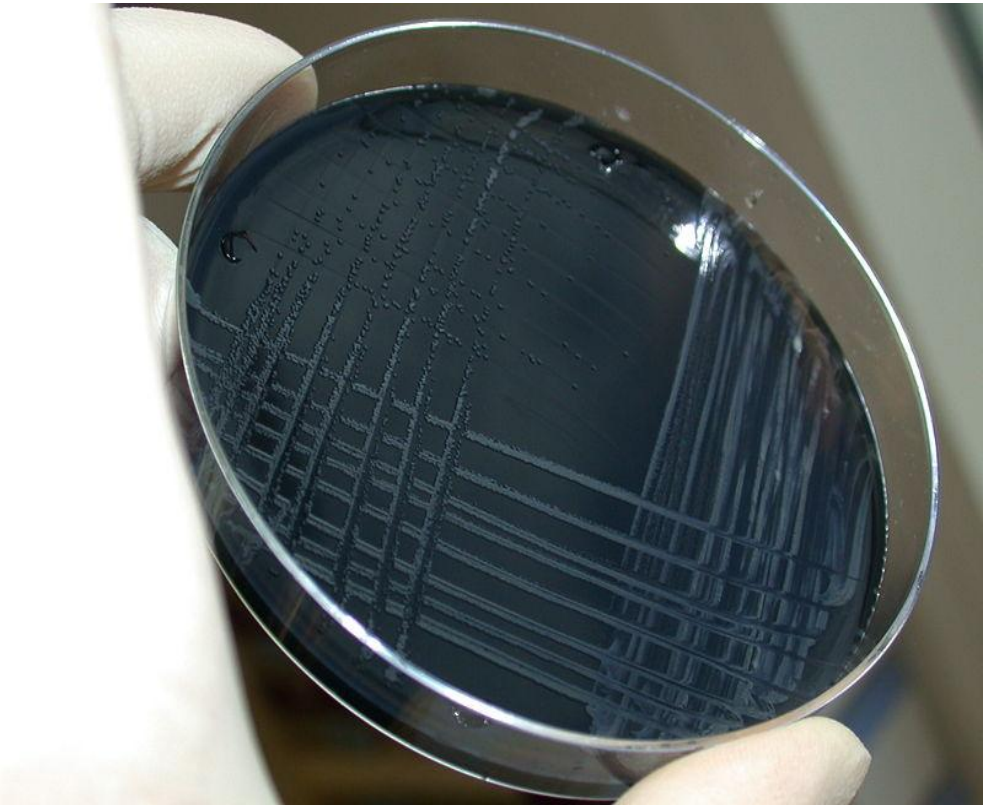
- Liquid mediums are often mixed with agar and poured into petri dishes to solidify. These agar plates provide a solid medium on which microbes may be cultured.

Types of Growth Media

- **Nutrient media**
  - ***Undefined media*** (also known as *basal* or *complex media*)
  - ***Defined media*** (also known as chemical defined media)
  - ***Differential medium*** some sort of indicator, typically a dye, is added, that allows for the differentiation of particular chemical reactions occurring during growth

Types of Growth Media

- **Selective media** (are used for the growth of only select microorganisms)

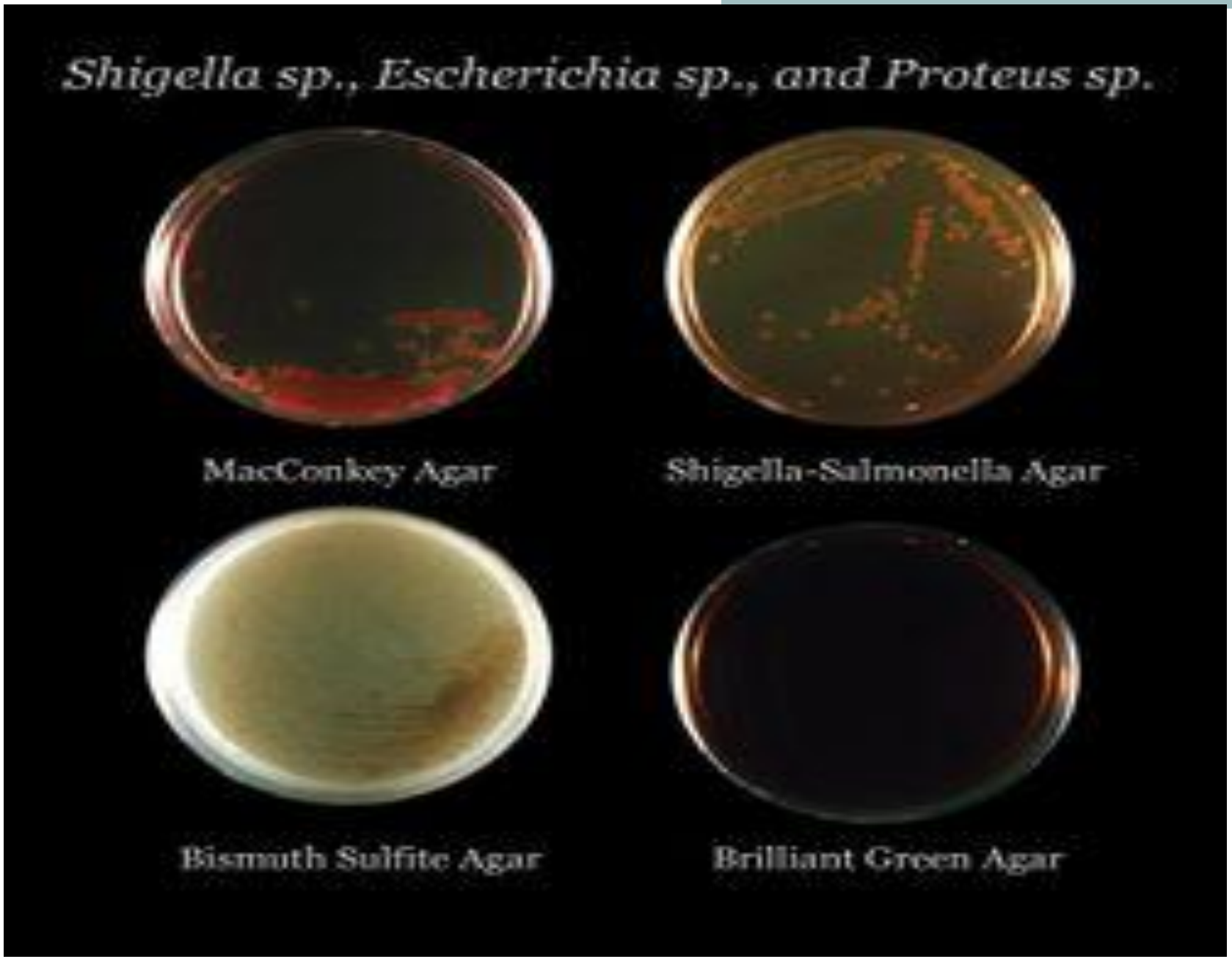


Blood-free, charcoal-based selective medium agar (CSM) for isolation of *Campylobacter*

Types of Growth Media

- **Differential media** or *indicator media* distinguish one microorganism type from another growing on the same media (MacConkey's, Nagler's medium)

  This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as **neutral red**, **phenol red**, **eosin y**, **or** **methylene blue**)


Shigella sp., Escherichia sp., and Proteus sp.
MacConkey Agar
Shigella-Salmonella Agar
Bismuth Sulfite Agar
Brilliant Green Agar

Types of Growth Media

- **Enriched media** contain the nutrients required to support the growth of a wide variety of organisms
  - *Blood agar* is an enriched medium in which nutritionally rich whole blood supplements the basic nutrients.
  - *Chocolate agar* is enriched with heat-treated blood (40-45°C), which turns brown and gives the medium the color for which it is named.

Blood agar plates are often used to diagnose infection. On the right is a positive ***Staphylococcus*** infection; on the left a positive ***Streptococcus*** culture.

Types of Growth Media

- **Transport media** used for the temporary storage of specimens being transported to the laboratory for cultivation. Transport media typically contain only buffers and salt (Stuart's medium for gonococci, buffeerd glycerol saline for enteric bacilli).
- **Indicator media** contain an indicator which chainges colour when a bacterium grows in them (Bismuth sulphite media(S.typhi), potassium tellurite(diphteria bacilli).

Types of Growth Media

- **Sugar Media** used for sugar fermentation (Hiss'serum sugars)
  - The sugar media consist of 1% of the sugar in peptone water along with an appropriate indicator
  - Durham's tube is kept inverted in the sugar tube to detect gas production
- **Anaerobic media** are used to grow anaerobic organisms (Robertson's cooked meat medium)

- Isolation of bacteria forms a very significant step in the diagnosis and management of the illness.
- Isolation of bacteria involves various steps –
- z Specimen collection
- z Preservation and transportation of specimen
- z Microscopic examination of sample
- z Various methods used for isolation of bacteria

- Common specimens include urine, faeces, wound swabs, throat swabs, vaginal swabs, sputum, and blood. Less common, but important specimens include cerebrospinal fluid, pleural fluid, joint aspirates, tissue, bone and prosthetic material (e.g. line tips).

- It is preferred to obtain the samples for bacteriological culture before antibiotic therapy is started. This maximizes the sensitivity of the investigations and reduces false-negative results.

- Specimens must be accurately labelled and accompanied by a properly completed requisition form, indicating the nature of the specimen, the date of sample collection, relevant clinical information, the investigations required, and details of antibiotic therapy, if any.

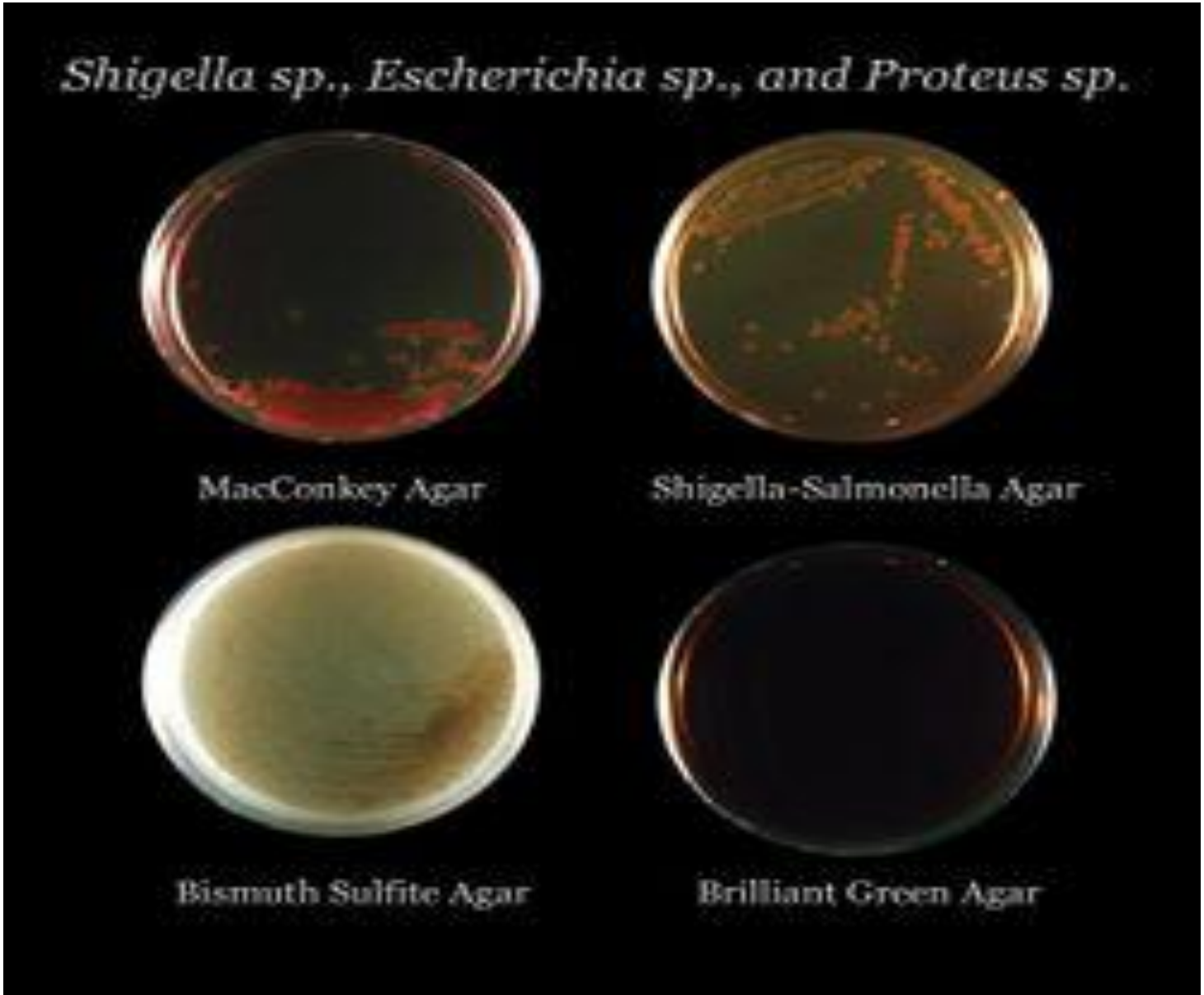
- Specimens should be transported as soon as possible to the laboratory. In case a delay is anticipated the specimen should be stored at 4° C.
- Immediate transport is necessary in order to:
- (i) Preserve the viability of the 'delicate' bacteria, such as Streptococcus pneumoniae or Haemophilus influenzae (delays in processing can cause false-negative culture results);

- (ii) Minimize the multiplication of bacteria (e.g. coliforms) within specimens before they reach the laboratory. In particular urine and other specimens that utilize a semiquantitative culture technique for their detection, as delays in transport can give rise to falsely high bacterial counts when the specimen is processed.

CULTURE ON SOLID MEDIA

- The principal method for the detection of bacteria from clinical specimens is by culture on solid culture media. Bacteria grow on the surface of culture media to produce distinct colonies.

- Different bacteria produce different but characteristic colonies, allowing for early presumptive identification and easy identification of mixed cultures.
- There are many different types of culture media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Method of inoculating the solid culture media

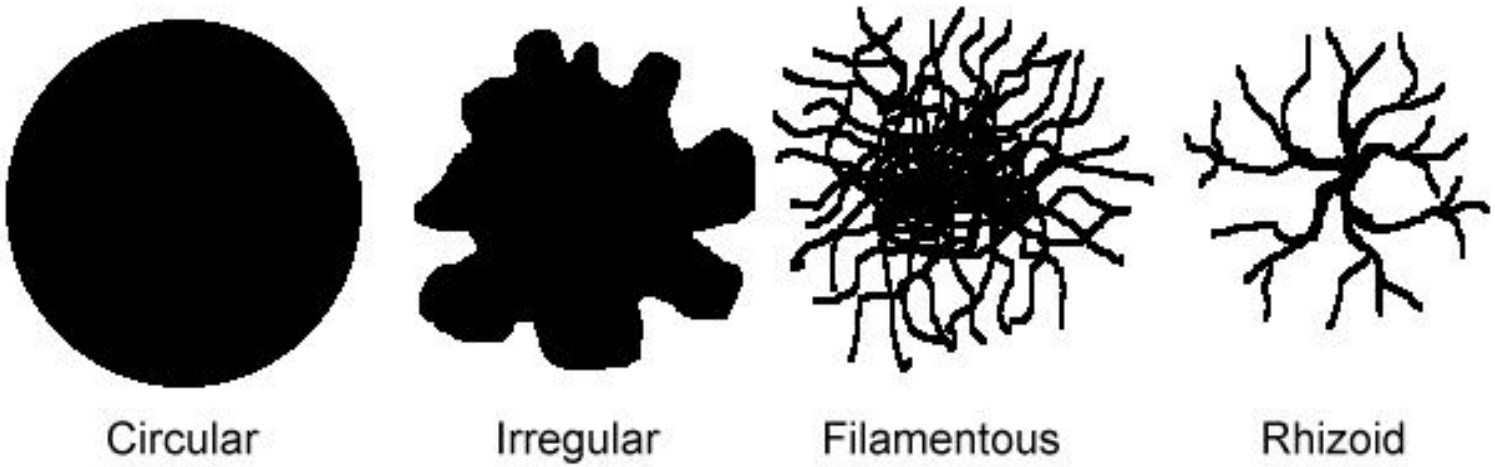
- For obtaining the isolated colonies streaking method is used, the most common method of inoculating an agar plate is streaking.

- In this method single bacterial cells get isolated by the streaking, and when the plate is incubated, forming discrete colonies that will have started from just one bacterium each

Colony Morphology of Bacteria

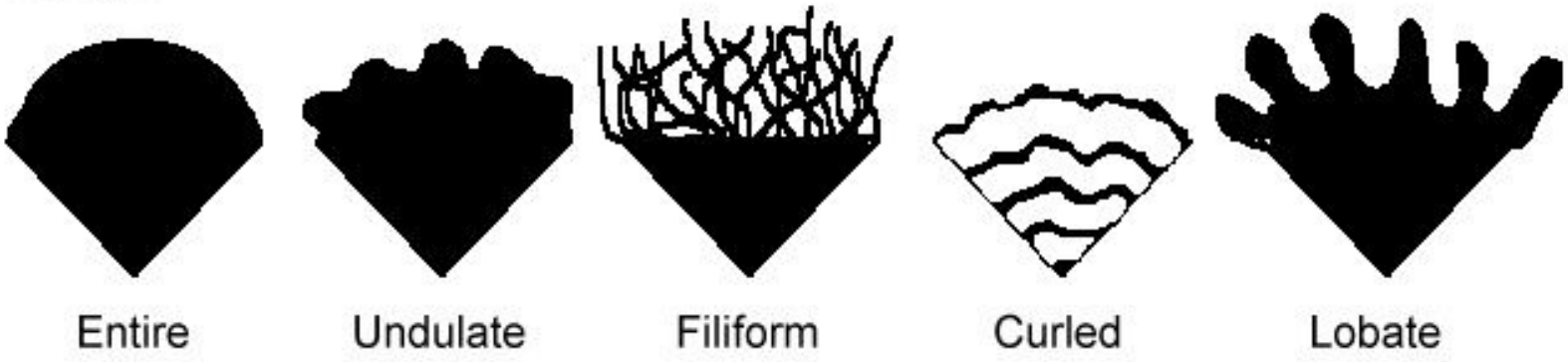
- Bacteria grow on solid media as colonies. A colony is defined as a visible mass of microorganisms all originating from a single mother cell. Key features of these bacterial colonies serve as an important criteria for their identification.

Form



Elevation

Margin



1. **Form of the bacterial colony**: – The form refers to the shape of the colony. These forms represent the most common colony shapes you are likely to encounter. e.g. Circular, Irregular, Filamentous, Rhizoid etc.
2. **Elevation of bacterial colony**: This describes the “side view” of a colony. These are the most common. e.g. **Flat, raised, umbonate (having a knobby protuberance), Crateriform, Convex, Pulvinate (Cushion-shaped)**

- **Margin of bacterial colony**: The margin or edge of a colony may be an important characteristic in identifying an organisms. Common examples are **Entire (smooth), irregular, Undulate (wavy), Lobate, Curled, Filiform** etc. Colonies that are irregular in shape and/or have irregular margins are likely to be motile organisms.

1. **Size of the bacterial colony**: The size of the colony can be a useful characteristic for identification. The diameter of a representative colony may be measured in millimeters or described in relative terms such as pin point, small, medium, large. Colonies larger than about 5 mm are likely to be motile organisms.

- **Appearance of the colony surface**: Bacterial colonies are frequently shiny and smooth in appearance. Other surface descriptions might be: dull (opposite of glistening), veined, rough, wrinkled (or shriveled), glistening.

- **Mixed growth of mucoid Lactose fermenting colonies and NLF colonies in MacConkey Agar**

- Color of the colonies (pigmentation): Some bacteria produce pigment when they grow in the medium e.g., green pigment produces by Pseudomonas aeruginosa, buff colored colonies of Mycobacterium tuberculosis in L.J medium, red colored colonies of Serratia marcescens.

- Opacity of the bacterial colony: Is the colony transparent (clear), opaque (not transparent or clear), translucent (almost clear, but distorted vision–like looking through frosted glass), iridescent (changing colors in reflected light).