Question 1: Explain the following terms (any nine): (2×9=18) (i) Generation time (ii) S layer (iii) L form (iv) VBNC (v) Enriched medium (vi) Pseudomurein (vii) Spheroplast (viii) Hopanoids (ix) Porins (x) Mesosomes

Explanation of Terms:

- **(i) Generation time:**
    - Generation time, also known as doubling time, is the time required for a population of bacteria to double in number during the exponential (log) phase of growth.
    - It is a characteristic property of a specific bacterial species under optimal growth conditions (temperature, pH, nutrients).
    - For example, *Escherichia coli* has a generation time of approximately 20-30 minutes under ideal laboratory conditions, while some slow-growing bacteria can have generation times of several hours or even days.
- **(ii) S layer:**
    - The S-layer (surface layer) is a regularly structured, two-dimensional array of protein or glycoprotein subunits that completely covers the outer surface of many bacteria and most archaea.
    - It serves as the outermost cell envelope component in many prokaryotes.
    - Functions include providing structural integrity, acting as a selective molecular sieve, participating in cell adhesion, protecting against host defenses (e.g., phagocytosis), and acting as a virulence factor.
- **(iii) L form:**
    - L-forms (also known as L-phase bacteria or cell wall-deficient bacteria) are bacterial variants that have lost most or all of their

cell wall, either spontaneously or due to exposure to cell wall-targeting agents (like antibiotics or lysozyme).

- They are typically spherical and osmotically fragile, requiring isotonic environments for survival.
- L-forms can be stable (unable to revert to walled form) or unstable (can revert). They are of medical interest as they can be resistant to antibiotics targeting the cell wall and may persist in chronic infections.

- **(iv) VBNC:**
  - VBNC stands for "Viable But Non-Culturable." It refers to a physiological state that some bacteria can enter under environmental stress (e.g., nutrient starvation, temperature extremes, osmotic shock, disinfectant exposure).
  - In the VBNC state, bacteria lose their ability to grow and form colonies on standard microbiological culture media, making them undetectable by routine laboratory methods.
  - However, they are still metabolically active and retain their viability, including the potential to regain culturability (resuscitate) and cause infection once favorable conditions return. This state poses a challenge for public health and food safety.
- **(v) Enriched medium:**
  - An enriched medium is a type of culture medium that contains additional nutrients or growth factors (such as blood, serum, vitamins, or specific amino acids) that are required by fastidious (nutritionally demanding) microorganisms.
  - These media are designed to support the growth of a wide variety of organisms, including those that may not grow on basic or general-purpose media.

- Examples include Blood Agar and Chocolate Agar, used for cultivating pathogenic bacteria.

- **(vi) Pseudomurein:**
  - Pseudomurein (also known as pseudopeptidoglycan) is a major component of the cell wall in some species of Archaea.
  - It is structurally similar to bacterial peptidoglycan but differs in its chemical composition.
  - Instead of N-acetylmuramic acid, it contains N-acetyltalosaminuronic acid, and the glycosidic bonds are β-1,3 linkages (not β-1,4 as in peptidoglycan). Also, the peptide cross-links contain L-amino acids, not D-amino acids.
  - This difference in structure makes archaeal cell walls insensitive to lysozyme and penicillin, which target peptidoglycan.

- **(vii) Spheroplast:**
  - A spheroplast is a bacterial cell (typically Gram-negative) that has been treated to remove most of its cell wall, but still retains its outer membrane.
  - They are spherical in shape and osmotically fragile, meaning they will lyse if placed in a hypotonic solution.
  - Spheroplasts are often generated in laboratories using enzymes like lysozyme and/or antibiotics like penicillin in an isotonic solution, and are used in various genetic manipulation techniques, such as transformation.

- **(viii) Hopanoids:**
  - Hopanoids are pentacyclic triterpenoid compounds found in the cell membranes of many bacteria.

- They are structurally similar to sterols (like cholesterol) found in eukaryotic cell membranes.
- Like sterols, hopanoids play a role in modulating membrane fluidity and permeability, especially in bacteria that lack sterols. They help to strengthen the membrane and allow bacteria to adapt to various environmental stresses.

- **(ix) Porins:**
  - Porins are barrel-shaped integral membrane proteins found primarily in the outer membrane of Gram-negative bacteria, as well as in the outer membranes of mitochondria and chloroplasts.
  - They form water-filled channels or pores that allow the passive diffusion of small, hydrophilic molecules (e.g., sugars, amino acids, ions, antibiotics) across the outer membrane, while excluding larger or hydrophobic molecules.
  - Porins are crucial for nutrient uptake and can also act as receptors for bacteriophages and bacteriocins.

- **(x) Mesosomes:**
  - Mesosomes are invaginations (infoldings) of the prokaryotic cell plasma membrane that were once thought to be distinct organelles involved in various cellular processes.
  - However, based on electron microscopy studies, they are now generally considered to be **artifacts** of chemical fixation methods used in preparing samples for electron microscopy, rather than true cellular structures.
  - Historically, they were hypothesized to be involved in DNA replication and segregation, cell division (septum formation), and respiration, but these functions are now attributed to the plasma membrane itself.

Question 2: (a) Differentiate between the following (any four): (4×4=16) (i) Spread plate and Pour plate method (ii) Gram-positive and Gram-negative Cell wall (iii) Synthetic and Complex media (iv) Polyphosphate and PHB granules (v) Archaebacterial and Eubacterial membranes (b) What are carboxysomes ? (2)

(a) Differentiation:

- **(i) Spread plate and Pour plate method:**
    - **Spread Plate Method:**
        - A small volume (typically 0.1 mL) of a diluted microbial sample is placed on the surface of a solidified agar medium in a Petri dish.
        - The sample is then evenly spread over the agar surface using a sterile bent glass rod (spreader).
        - Colonies grow only on the surface of the agar.
        - Used for viable cell counting, isolating individual colonies, and when oxygen exposure is important for growth.
        - Relatively less prone to heat damage for heat-sensitive microbes.
    - **Pour Plate Method:**
        - A small volume of a diluted microbial sample is added to an empty, sterile Petri dish.
        - Molten (cooled to 45-50°C) agar medium is then poured into the dish, mixed with the sample, and allowed to solidify.
        - Colonies develop both within the agar (subsurface) and on the surface.

- Used for viable cell counting, especially for obligate or facultative anaerobes, as some colonies grow embedded in the agar, protected from high oxygen levels.
- Heat-sensitive microbes might be damaged by the molten agar.

- **(ii) Gram-positive and Gram-negative Cell wall:**
  - **Gram-Positive Cell Wall:**
    - **Thickness:** Very thick (20-80 nm).
    - **Peptidoglycan:** Consists of a thick, multi-layered peptidoglycan (murein) layer (50-90% of cell wall weight).
    - **Outer Membrane:** Absent.
    - **Teichoic Acids:** Present and characteristic. These are polymers of glycerol phosphate or ribitol phosphate, often linked to the peptidoglycan and/or plasma membrane (lipoteichoic acids). They contribute to the negative charge of the cell wall and are involved in cell adhesion and antigenicity.
    - **Periplasmic Space:** Very small or absent.
    - **LPS (Lipopolysaccharide):** Absent.
    - **Staining:** Retains the crystal violet-iodine complex, appearing purple/blue after Gram staining.
  - **Gram-Negative Cell Wall:**
    - **Thickness:** Thin (10-15 nm).
    - **Peptidoglycan:** Consists of a thin, single or double layer of peptidoglycan (5-10% of cell wall weight).
    - **Outer Membrane:** Present outside the peptidoglycan layer. This outer membrane is unique and contains

lipopolysaccharide (LPS), phospholipids, and proteins (including porins).

- **Teichoic Acids:** Absent.
- **Periplasmic Space:** Large and prominent, located between the plasma membrane and the outer membrane. It contains enzymes and transport proteins.
- **LPS (Lipopolysaccharide):** Present in the outer leaflet of the outer membrane. LPS consists of Lipid A (an endotoxin), core polysaccharide, and O-antigen (a major antigen).
- **Staining:** Does not retain the crystal violet-iodine complex and is counterstained by safranin, appearing pink/red after Gram staining.

- **(iii) Synthetic and Complex media:**
  - **Synthetic Medium (Defined Medium):**
    - **Composition:** All chemical components are known precisely and are added in exact, quantified amounts. The exact chemical formula of every ingredient is known.
    - **Reproducibility:** Highly reproducible results due to precise composition.
    - **Use:** Used for research studies where precise control over nutrient conditions is required, such as metabolic studies or determining specific nutritional requirements of an organism.
    - **Growth:** Generally supports the growth of less fastidious organisms as all specific growth factors must be individually added.
  - **Complex Medium (Undefined Medium):**

- **Composition:** Contains ingredients whose exact chemical composition is not precisely known or varies from batch to batch. These often include extracts from plant or animal sources (e.g., yeast extract, beef extract, peptones).
- **Reproducibility:** Less reproducible than synthetic media due to variable composition.
- **Use:** Used for general cultivation of a wide variety of microorganisms, including fastidious ones, because it provides a rich blend of growth factors and nutrients. Common in routine laboratory work.
- **Growth:** Supports the growth of a broad range of organisms as it provides a complex mixture of amino acids, vitamins, and other growth factors.

- **(iv) Polyphosphate and PHB granules:**
  - **Polyphosphate Granules (Volutin Granules/Metachromatic Granules):**
    - **Composition:** Are accumulations of inorganic polyphosphate, which are linear polymers of orthophosphate residues linked by high-energy phosphoanhydride bonds.
    - **Function:** Serve as a reserve of inorganic phosphate for nucleic acid synthesis (DNA, RNA), phospholipid synthesis, and ATP synthesis. They can also store energy.
    - **Staining:** Stains red or blue with methylene blue (metachromatic staining).
    - **Location:** Found in the cytoplasm of various bacteria and some eukaryotic microorganisms.

- **PHB Granules (Poly-β-hydroxybutyrate Granules):**
  - **Composition:** Are accumulations of poly-β-hydroxybutyrate, a type of polyhydroxyalkanoate (PHA), which are polyesters of 3-hydroxybutyrate.
  - **Function:** Serve as a major carbon and energy storage compound in many bacteria when carbon is abundant but other nutrients (like nitrogen or phosphorus) are limited. They are biodegradable plastics.
  - **Staining:** Stains black with Sudan black B.
  - **Location:** Found as discrete inclusions in the cytoplasm of various bacteria.

- **(v) Archaebacterial and Eubacterial membranes:**
  - **Archaebacterial Membranes (Archaeal Membranes):**
    - **Lipid Linkage:** Lipids are linked to glycerol by **ether bonds** (C-O-C).
    - **Fatty Acid Structure:** Fatty acid chains are branched hydrocarbons (isoprenoids), not straight chains.
    - **Glycerol Linkage:** Glycerol is usually linked to two hydrocarbon chains at the Sn-2 and Sn-3 positions (glycerol diethers) or to two chains forming a single lipid monolayer (diglycerol tetraethers), enabling membrane stability at high temperatures.
    - **Membrane Type:** Can form both lipid bilayers and lipid monolayers. Monolayers are common in hyperthermophilic archaea, providing extreme heat stability.
    - **Sterols/Hopanoids:** Generally lack sterols, but some may contain hopanoids or structurally related compounds.

- **Eubacterial Membranes (Bacterial Membranes):**
  - **Lipid Linkage:** Lipids are linked to glycerol by **ester bonds** (C-O).
  - **Fatty Acid Structure:** Fatty acid chains are typically unbranched, straight-chain hydrocarbons.
  - **Glycerol Linkage:** Glycerol is linked to two fatty acid chains at the Sn-1 and Sn-2 positions (glycerol diesters), forming diglycerides.
  - **Membrane Type:** Always form lipid bilayers.
  - **Sterols/Hopanoids:** Generally lack sterols, but many contain hopanoids which function similarly to sterols.

(b) What are carboxysomes?

- **Carboxysomes:**
  - Carboxysomes are polyhedral proteinaceous (protein-shelled) microcompartments found in the cytoplasm of many autotrophic bacteria (e.g., cyanobacteria, nitrifying bacteria, some chemoautotrophs).
  - They are essentially specialized bacterial organelles that encapsulate enzymes involved in carbon dioxide fixation.
  - **Function:** Their primary function is to enhance the efficiency of carbon dioxide fixation by concentrating the enzyme RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase) and its substrate, CO2, within the compartment. They also contain carbonic anhydrase, which rapidly converts bicarbonate (HCO_3^-) into CO2, thereby creating a high concentration of CO2 around RuBisCO and minimizing its wasteful oxygenase activity.
  - This concentration mechanism helps to overcome the kinetic limitations of RuBisCO and improves photosynthetic or chemosynthetic efficiency.

Question 3: (a) Write short notes (any four): (4×4=16) (i) Asexual reproduction in bacteria (ii) Patterns of flagella distribution in bacteria (iii) Cultivation of anaerobic bacteria (iv) Steps in formation of bacterial endospore (v) Methods of maintenance and preservation of bacterial cultures (b) How do plasmids differ from chromosomes? (2)

(a) Short Notes:

- **(i) Asexual reproduction in bacteria:**
    - Asexual reproduction is the primary mode of multiplication in bacteria, leading to the rapid increase in population size without the involvement of gametes or genetic recombination between different individuals.
    - **Binary Fission:** The most common method. In this process, a single bacterial cell grows in size, replicates its genetic material (chromosome), and then divides into two identical daughter cells.
        i. **Cell Elongation:** The cell elongates, and the chromosome replicates.
        ii. **Chromosome Segregation:** The two identical chromosomes move to opposite ends of the elongating cell.
        iii. **Septum Formation:** A new cell wall and cell membrane (septum) begin to grow inward from the periphery of the cell, dividing it into two halves.
        iv. **Cell Separation:** The septum completely divides the cell, resulting in two separate, genetically identical daughter cells.
    - **Budding:** Some bacteria, particularly certain aquatic species, reproduce by budding, where a smaller daughter cell grows out from the parent cell and then detaches.

- **Fragmentation:** Filamentous bacteria may reproduce by fragmentation, where a filament breaks into several pieces, each capable of growing into a new filament.
- **Conidiospores:** Some filamentous bacteria (e.g., *Streptomyces*) produce asexual spores called conidiospores at the tips of aerial hyphae.
- This rapid asexual reproduction allows bacterial populations to quickly adapt to favorable environmental conditions and exploit new resources.

- **(ii) Patterns of flagella distribution in bacteria:**
  - Bacterial flagella are whip-like appendages used for motility, and their arrangement on the bacterial cell surface is a key characteristic used in classification.
  - **Monotrichous:** A single flagellum located at one pole (end) of the cell.
    - Example: *Vibrio cholerae*, *Pseudomonas aeruginosa*.
  - **Amphitrichous:** A single flagellum at each of the two opposite poles of the cell.
    - Example: *Spirillum minor*, *Alcaligenes faecalis*.
  - **Lophotrichous:** A tuft or cluster of multiple flagella at one or both poles of the cell.
    - Example: *Spirillum serpens*, *Helicobacter pylori*.
  - **Peritrichous:** Numerous flagella distributed uniformly over the entire surface of the cell. These flagella rotate in a coordinated manner to allow movement.
    - Example: *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*.
  - **Atrichous:** No flagella present. These bacteria are non-motile.

        - Example: *Klebsiella pneumoniae*, *Shigella dysenteriae*.
    - The specific arrangement influences the swimming patterns and motility characteristics of the bacteria.

- **(iii) Cultivation of anaerobic bacteria:**
    - Cultivation of anaerobic bacteria, which cannot grow in the presence of oxygen (or are even harmed by it), requires specialized techniques to create and maintain an oxygen-free environment.
    - **Reducing Agents in Media:** Incorporating reducing agents into the culture medium to chemically remove oxygen. Examples include thioglycollate, cysteine, or sodium sulfide. Thioglycollate broth contains sodium thioglycollate, which reduces oxygen and establishes an oxygen gradient, with higher oxygen at the top and anaerobic conditions deeper in the tube.
    - **Anaerobic Jars/Chambers:**
        - **Anaerobic Jar:** A sealed container (e.g., GasPak system) where oxygen is removed by chemical reactions. Hydrogen and carbon dioxide are produced, which react with a palladium catalyst to form water, consuming oxygen. An indicator strip (e.g., methylene blue, resazurin) confirms anaerobic conditions.
        - **Anaerobic Chamber (Glove Box):** A sealed, airtight workstation equipped with an inert gas atmosphere (e.g., nitrogen, hydrogen, carbon dioxide) and an airlock for introducing materials. Allows for direct manipulation of cultures in an oxygen-free environment.
    - **Oxygen-Free Gas Flushing:** Sparging media and containers with oxygen-free gases (e.g., nitrogen or argon) before and during inoculation.

- **Deep Agar Stabs:** Inoculating bacteria into the bottom of deep agar tubes where oxygen penetration is minimal.
- **Roll Tubes (Hungate Tubes):** Test tubes with culture medium solidified on the inner surface, flushed with anaerobic gas, and sealed with a stopper. Used for cultivating strict anaerobes.
- These methods are crucial for isolating, culturing, and studying diverse anaerobic microorganisms, many of which play important roles in ecosystems and human health.

- **(iv) Steps in formation of bacterial endospore:**
  - Bacterial endospores are highly resistant, dormant structures formed by certain Gram-positive bacteria (e.g., *Bacillus* and *Clostridium*) in response to unfavorable environmental conditions (e.g., nutrient depletion, desiccation). This process is called sporulation.
  - **Steps of Sporulation (typically 7 stages):**
    v. **Axial Filament Formation:** The cell's DNA replicates, and the two chromosomes move to opposite ends of the cell, forming an axial filament of nucleoid material.

    vi. **Septum Formation:** The plasma membrane invaginates inward, forming an asymmetric septum near one pole of the cell, separating a smaller prespore compartment from the larger mother cell compartment.

    vii. **Engulfment:** The mother cell membrane engulfs the prespore, enclosing it within two membranes. The prespore is now a protoplast with an inner and outer membrane, surrounded by cytoplasm from the mother cell.

    viii. **Cortex Formation:** A thick layer of peptidoglycan, called the cortex, is laid down in the space between the inner and outer spore membranes.

ix. **Spore Coat Formation:** Layers of specialized proteins (spore coat proteins) are synthesized in the mother cell cytoplasm and deposited on the outer surface of the outer spore membrane, forming a protective spore coat.

x. **Maturation and Dehydration:** The spore matures, and its core undergoes significant dehydration. Calcium dipicolinate accumulates in the core, stabilizing DNA and proteins. Small acid-soluble spore proteins (SASPs) are synthesized and bind to DNA.

xi. **Lysis of Mother Cell:** The mother cell lyses, releasing the mature, free endospore into the environment.

- Endospores are extremely resistant to heat, radiation, chemicals, and desiccation, allowing the bacteria to survive adverse conditions for extended periods.

- **(v) Methods of maintenance and preservation of bacterial cultures:**
  - Maintaining bacterial cultures in a viable, pure, and genetically stable state for extended periods is crucial for research, industrial, and diagnostic purposes.
  - **Short-Term Preservation:**

xii. **Refrigeration (4°C):** For many non-fastidious bacteria, growing cultures on agar slants or in broth and then storing them in a refrigerator (4°C) can keep them viable for weeks to a few months. Regular subculturing (transfer to fresh media) is required.

xiii. **Paraffin Overlay (Mineral Oil Overlay):** Covering agar slant cultures with sterile mineral oil creates an anaerobic environment and reduces desiccation, extending viability for several months to a year.

  - **Long-Term Preservation (Genetic Stability and Viability):**

xiv. **Cryopreservation (Deep Freezing):**

- Involves suspending bacterial cells in a cryoprotective agent (e.g., 10-20% glycerol or DMSO - dimethyl sulfoxide) to prevent ice crystal formation that could damage cells.
- Cultures are then frozen rapidly and stored at very low temperatures, typically -70°C in ultra-low freezers or -196°C in liquid nitrogen.
- This method can preserve cultures for many years (decades).

xv. **Lyophilization (Freeze-Drying):**

- Involves freezing a bacterial suspension in a cryoprotective medium and then removing the water by sublimation under vacuum.
- This results in a dry powder of viable cells.
- Lyophilized cultures are sealed in ampoules and stored at 4°C or room temperature.
- This method is excellent for long-term preservation, often maintaining viability for decades, with minimal genetic change. Rehydration with sterile broth or water reactivates the cells.

xvi. **Storage in Sterile Water:** Some bacteria can be stored for extended periods in sterile distilled water at room temperature, although viability may decrease over time.

(b) How do plasmids differ from chromosomes?

- **Plasmids:**

- **Nature:** Extrachromosomal, autonomously replicating DNA molecules.
- **Size:** Relatively small, typically ranging from a few kilobases to hundreds of kilobases.
- **Shape:** Usually circular, double-stranded DNA.
- **Essentiality:** Generally non-essential for basic cell survival and reproduction under normal conditions.
- **Genes Carried:** Often carry genes that provide advantageous traits, such as antibiotic resistance, heavy metal resistance, virulence factors, or metabolic pathways for degrading unusual compounds.
- **Number:** Can be present in multiple copies per cell (high-copy number) or a few copies (low-copy number).
- **Inheritance:** Replicate independently of the main chromosome and are passed on to daughter cells during cell division. Can also be transferred horizontally between bacteria via conjugation.
- **Location:** Found in the cytoplasm of prokaryotic cells.

- **Chromosomes:**
  - **Nature:** The main genetic material of an organism.
  - **Size:** Large, typically millions of base pairs long.
  - **Shape:** Usually a single, circular, double-stranded DNA molecule in prokaryotes (linear in eukaryotes). Some bacteria may have multiple circular chromosomes.
  - **Essentiality:** Essential for the survival and reproduction of the cell, carrying all the genes required for basic cellular functions (e.g., metabolism, replication, cell structure).

- **Genes Carried:** Carry housekeeping genes and genes encoding essential proteins.
- **Number:** Typically one per cell in prokaryotes (though replication leads to multiple copies before division).
- **Inheritance:** Replicated and precisely segregated to daughter cells during cell division as part of the primary genome.
- **Location:** Located in the nucleoid region of the cytoplasm in prokaryotic cells (or within the nucleus in eukaryotes).

Question 4: (a) Give one example of each of the following (any eight): (1×8=8) (i) Selective and differential medium (ii) Bacteria with proteinaceous capsule (iii) Giant bacteria (iv) Bacteria harbouring sulphur granules (v) Magnetotactic bacteria (vi) Spiral shaped bacteria (vii) Bacteria containing more than 1 chromosome (viii) Gram positive rods (ix) Endospore forming bacteria (b) Diagrammatically describe the phases of a typical bacterial growth curve. (5) (c) Discuss the use of enrichment cultures in isolating microbes. (3) (d) What are the functions of pili? (2)

(a) Examples of Microbes:

- (i) Selective and differential medium: **MacConkey Agar**
- (ii) Bacteria with proteinaceous capsule: *Bacillus anthracis*
- (iii) Giant bacteria: *Thiomargarita namibiensis* (or *Epulopiscium fishelsoni*)
- (iv) Bacteria harbouring sulphur granules: *Thioploca* species (or *Beggiatoa* species, Purple sulfur bacteria)
- (v) Magnetotactic bacteria: *Magnetospirillum magnetotacticum*
- (vi) Spiral shaped bacteria: *Spirillum minor* (or *Treponema pallidum*, *Borrelia burgdorferi*)
- (vii) Bacteria containing more than 1 chromosome: *Vibrio cholerae* (has two chromosomes)

- (viii) Gram positive rods: *Bacillus subtilis* (or *Clostridium tetani*, *Listeria monocytogenes*)
- (ix) Endospore forming bacteria: *Clostridium botulinum* (or *Bacillus cereus*)

(b) Diagrammatically describe the phases of a typical bacterial growth curve. I cannot generate diagrams. However, I can describe the phases of a typical bacterial growth curve in detail.

- **Phases of a Typical Bacterial Growth Curve:** When a population of bacteria is inoculated into a fresh batch of nutrient medium and incubated under optimal conditions, its growth (increase in cell number) follows a characteristic pattern over time, which can be plotted as a growth curve. The curve typically consists of four distinct phases:

b. **Lag Phase:**

- **Description:** Immediately after inoculation, there is no immediate increase in cell number. This initial period is characterized by intense metabolic activity where individual cells are adjusting to the new environment.
- **Activities:** Bacteria synthesize enzymes, ribosomes, ATP, and other molecules necessary for rapid growth. They may also repair any cellular damage from the previous environment. Cell size may increase, but cell division is minimal or absent.
- **Duration:** The length of the lag phase varies depending on the previous growth conditions, the age of the inoculum, the nature of the medium, and the bacterial species.

c. **Log Phase (Exponential Growth Phase):**

- **Description:** During this phase, bacteria multiply at an exponential rate. The number of cells doubles at regular

intervals (generation time). This is the period of most vigorous growth.

- **Activities:** Cells are actively dividing by binary fission. The population is metabolically uniform, and cells are typically at their healthiest. The growth rate is constant and maximal under the given conditions.
- **Rate:** The plot of the logarithm of cell number versus time yields a straight line during this phase.

d. **Stationary Phase:**

- **Description:** The rate of cell division slows down significantly, and the number of new cells produced equals the number of cells dying. The total viable cell count remains relatively constant, resulting in a plateau on the growth curve.
- **Causes:** This phase is reached due to the depletion of essential nutrients, accumulation of toxic waste products, or changes in environmental conditions such as pH or oxygen levels.
- **Activities:** Cells enter a survival mode, often becoming smaller, undergoing metabolic shifts, and sometimes producing secondary metabolites (e.g., antibiotics, enzymes) or resistant structures (e.g., endospores) in response to stress.

e. **Death Phase (Decline Phase):**

- **Description:** The number of viable cells decreases exponentially. The rate of cell death exceeds the rate of cell division.
- **Causes:** Continued nutrient depletion and accumulation of toxic waste products lead to a harsh environment that becomes lethal for the majority of the population.

- **Activities:** Cells undergo irreversible damage and lysis. The rate of death can vary depending on the bacterial species and the severity of the unfavorable conditions. However, a small fraction of the population may survive for an extended period, possibly by utilizing nutrients released from dead cells.

(c) Discuss the use of enrichment cultures in isolating microbes.

- **Enrichment Cultures:**
  - Enrichment culture is a powerful microbiological technique used to selectively increase the numbers of specific types of microorganisms from a mixed environmental sample (e.g., soil, water, clinical specimens) by providing highly selective growth conditions.
  - The goal is to favor the growth of desired microbes while suppressing the growth of undesired ones, making the isolation of specific organisms much easier.

- **How it Works (Principles):**
  f. **Selective Medium:** A medium is formulated with specific nutrients, inhibitors, or physical conditions that only allow the target microorganism (or a specific metabolic group) to thrive. This creates a competitive advantage for the desired organism.

  g. **Environmental Conditions:** Incubation conditions (e.g., temperature, pH, aeration, light, redox potential) are carefully controlled to mimic or optimize the preferred conditions of the target microbe.

  h. **Inoculation:** A small amount of the environmental sample, containing a diverse microbial community, is inoculated into the enrichment medium.

  i. **Incubation:** The culture is incubated, allowing the desired organisms to grow to high numbers.

j. **Subculturing:** After sufficient growth, a small portion of the enrichment culture is transferred to a fresh selective medium (sometimes to a more inhibitory selective medium or a solid medium) to further increase purity and eventually isolate individual colonies. This "serial dilution" step helps dilute out non-target organisms.

- **Examples of Application:**
  - **Nitrogen-fixing bacteria:** To isolate nitrogen-fixing bacteria (e.g., *Azotobacter*), a medium lacking a fixed nitrogen source (e.g., ammonium, nitrate) is used. Only organisms capable of fixing atmospheric nitrogen will grow.
  - **Cellulose-degrading bacteria:** To isolate bacteria that degrade cellulose, a medium where cellulose is the sole carbon source is used.
  - **Thermophilic bacteria:** Incubation at high temperatures (e.g., 60-80°C) selects for thermophilic organisms from a soil sample.
  - **Sulfate-reducing bacteria:** Using a medium with sulfate as the sole electron acceptor and strictly anaerobic conditions will enrich for sulfate reducers.
  - **Pathogen Isolation:** Many clinical laboratories use enrichment broths (e.g., selenite broth for *Salmonella*) to increase the numbers of specific pathogens from fecal samples before plating on selective agar.
- **Significance:**
  - **Isolation of Rare Microbes:** Enables the isolation of microorganisms that are rare in their natural habitat or difficult to culture using general methods.
  - **Study of Metabolic Diversity:** Helps in understanding the metabolic capabilities and ecological roles of various microbial groups.

- **Industrial Applications:** Crucial for isolating microbes with specific industrial applications (e.g., antibiotic producers, biodegraders).
- **Environmental Microbiology:** Essential for studying microbial communities and their functions in diverse environments.

(d) What are the functions of pili?

- **Pili (Fimbriae):**
  - Pili (singular: pilus), also known as fimbriae (singular: fimbria), are short, hair-like protein appendages that extend from the surface of many bacteria, particularly Gram-negative species. They are thinner and shorter than flagella and are not involved in motility (with the exception of twitching motility by type IV pili).
- **Primary Functions:**

k. **Adhesion/Attachment (Adherence):**

- This is the most well-known and crucial function. Pili act as adhesins, allowing bacteria to specifically bind to host cell surfaces, tissues, or inanimate objects. This is a critical step in colonization and initiation of infection (virulence factor).
- For example, *Escherichia coli* uses pili to adhere to the urinary tract lining, and *Neisseria gonorrhoeae* uses them to attach to host epithelial cells.

l. **Biofilm Formation:**

- By facilitating initial attachment to surfaces, pili play a significant role in the formation of biofilms, which are communities of microorganisms encased in an extracellular polymeric substance. Biofilms are important in chronic infections and surface colonization.

m. **Conjugation (Sex Pili):**

- A specialized type of pilus, called the **sex pilus** or **F pilus**, is involved in bacterial conjugation. This pilus forms a bridge between a donor bacterium (carrying an F plasmid) and a recipient bacterium, allowing the transfer of genetic material (plasmids or parts of the chromosome).

n. **Twitching Motility (Type IV Pili):**

- Some bacteria with Type IV pili can exhibit a form of surface motility called "twitching motility." This occurs through the extension and retraction of the pilus, pulling the cell along a surface. This type of motility is important for biofilm formation, surface colonization, and pathogenesis in some bacteria (e.g., *Pseudomonas aeruginosa*).

o. **DNA Uptake (Transformation):**

- Type IV pili are also involved in natural transformation, the process by which some bacteria take up naked DNA from their environment, leading to genetic recombination.

p. **Antigenicity:**

- Pili proteins can be highly antigenic, recognized by the host immune system. However, some bacteria can undergo pilus phase variation (switching pilus types) to evade immune detection.

Question 5: (a) Diagrammatically explain the structure of gram-negative bacterial flagella. (5) (b) Discuss the different groups of bacteria based on their oxygen requirements citing suitable examples. (5) (c) What are the functions of a bacterial capsule? (4) (d) What is the significance of culture collections? Name any two microbial culture collection centres. (4)

(a) Diagrammatically explain the structure of gram-negative bacterial flagella. I cannot generate diagrams. However, I can provide a detailed explanation of the structure of a Gram-negative bacterial flagellum.

- **Structure of a Gram-Negative Bacterial Flagellum:** A bacterial flagellum is a complex, helical filament that extends from the cell surface and is responsible for bacterial motility (swimming). The structure is highly conserved across motile bacteria but differs significantly between Gram-positive and Gram-negative bacteria, particularly at the basal body.

  The flagellum of a Gram-negative bacterium consists of three main parts:

  q. **Filament:**

  - This is the longest and most visible part of the flagellum, extending outwards into the environment.
  - It is a rigid, hollow cylinder composed of thousands of identical protein subunits called **flagellin**.
  - The flagellin proteins are arranged helically around a hollow core.
  - The filament is responsible for propelling the cell by rotating like a propeller.

  r. **Hook:**

  - The hook is a short, curved, hollow structure located at the base of the filament, connecting the filament to the cell surface structures.
  - It acts as a universal joint, transmitting the rotational force generated by the basal body to the rigid filament, allowing the filament to rotate in various directions.
  - It is composed of a single type of protein called **Hook protein**.

s. **Basal Body:**

- This is the most complex part of the flagellum, embedded within the cell envelope. It functions as the rotary motor that drives the flagellum's rotation.
- In Gram-negative bacteria, the basal body consists of a central rod and a series of four rings that anchor the flagellum to the cell envelope layers:
    - **L Ring (Lipopolysaccharide Ring):** The outermost ring, associated with the **outer membrane** (specifically, it interacts with LPS).
    - **P Ring (Peptidoglycan Ring):** Located just beneath the L ring, embedded in the **peptidoglycan layer**.
    - **MS Ring (Membrane-Supramembrane Ring):** Embedded in the **cytoplasmic membrane (inner membrane)**. This ring is believed to be the motor component, where the rotation is generated.
    - **C Ring (Cytoplasmic Ring):** Located on the inner surface of the **cytoplasmic membrane**, extending into the cytoplasm. This ring interacts with motor proteins and proteins involved in flagellar assembly.
- **Motor (Mot) Proteins:** These proteins are associated with the MS and C rings in the cytoplasmic membrane. They generate the torque for flagellar rotation by utilizing the energy from the proton motive force (PMF) across the cytoplasmic membrane. Protons flow through these proteins, causing the MS and C rings (and thus the rod, hook, and filament) to rotate.
- **Switch (Fli) Proteins:** Located near the C ring, these proteins act as a switch, controlling the direction of

flagellar rotation (clockwise or counter-clockwise), which determines the bacterial swimming behavior (runs and tumbles).

In summary, the Gram-negative flagellum is a sophisticated molecular machine, with the filament acting as the propeller, the hook as the universal joint, and the basal body as the intricate motor embedded in the multiple layers of the Gram-negative cell envelope.

(b) Discuss the different groups of bacteria based on their oxygen requirements citing suitable examples.

- Bacteria exhibit diverse relationships with oxygen, leading to distinct metabolic strategies and ecological niches. Based on their oxygen requirements, bacteria can be classified into several groups:

a. **Obligate Aerobes:**

- **Description:** These bacteria absolutely require oxygen for growth. They use oxygen as the final electron acceptor in aerobic respiration to generate ATP.
- **Growth:** Grow only in the presence of oxygen, typically at the top of a liquid culture medium where oxygen concentration is highest.
- **Enzymes:** Possess enzymes like superoxide dismutase (SOD) and catalase (or peroxidase) to detoxify reactive oxygen species (ROS) like superoxide radicals and hydrogen peroxide, which are harmful byproducts of aerobic respiration.
- **Examples:** *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Bacillus subtilis*.

b. **Microaerophiles:**

- **Description:** These bacteria require oxygen for growth, but only in concentrations lower than that in the

atmosphere (typically 2-10% oxygen). Higher concentrations of oxygen can be inhibitory or even lethal.

- **Growth:** Grow in a narrow zone below the surface of a liquid culture, where oxygen levels are reduced.
- **Enzymes:** Have limited capacities to detoxify ROS compared to obligate aerobes.
- **Examples:** *Helicobacter pylori*, *Campylobacter jejuni*.

c. **Facultative Anaerobes:**

- **Description:** These bacteria can grow either in the presence or absence of oxygen. They prefer aerobic conditions because aerobic respiration yields more ATP, but they can switch to anaerobic respiration or fermentation if oxygen is unavailable.
- **Growth:** Grow best where oxygen is present but can also grow throughout the medium, though less efficiently, in anaerobic zones.
- **Enzymes:** Possess both SOD and catalase (or peroxidase).
- **Examples:** *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*.

d. **Aerotolerant Anaerobes:**

- **Description:** These bacteria do not use oxygen for metabolism and are strictly fermentative, but they can tolerate its presence and grow in its presence. They do not grow better with oxygen.
- **Growth:** Grow evenly throughout a liquid medium, independent of oxygen concentration.

- **Enzymes:** Possess SOD (to neutralize superoxide radicals) but typically lack catalase or peroxidase.
- **Examples:** *Streptococcus pyogenes*, *Enterococcus faecalis*.

e. **Obligate Anaerobes (Strict Anaerobes):**

- **Description:** These bacteria are killed by oxygen and can only grow in its complete absence. Oxygen is toxic to them because they lack or have very low levels of enzymes (SOD, catalase, peroxidase) needed to detoxify reactive oxygen species.
- **Growth:** Grow only at the bottom of a liquid culture medium or in strictly anaerobic environments.
- **Enzymes:** Lack SOD and catalase/peroxidase.
- **Examples:** *Clostridium botulinum*, *Clostridium tetani*, *Bacteroides fragilis*.

(c) What are the functions of a bacterial capsule?

- **Bacterial Capsule:**
  - A bacterial capsule is an outermost, gelatinous, sticky, and well-organized layer of polysaccharide (or sometimes polypeptide) that surrounds the cell wall of some bacteria. It is distinct from the slime layer, which is less organized and more loosely associated.
- **Key Functions of a Bacterial Capsule:**

a. **Protection Against Phagocytosis (Antiphagocytic Function):**

- This is one of the most important functions, especially for pathogenic bacteria. The capsule makes the bacterial cell more difficult for phagocytic immune cells (like

macrophages and neutrophils) to engulf and destroy. It effectively masks the underlying cell wall components that would normally be recognized as foreign by phagocytes, thus enhancing bacterial virulence.

- Example: *Streptococcus pneumoniae* (causes pneumonia), *Klebsiella pneumoniae*.

b. **Adherence/Attachment (Adhesion to Surfaces and Host Tissues):**

- The sticky, gummy nature of the capsule allows bacteria to adhere strongly to various surfaces, including host tissues (e.g., mucous membranes, teeth), medical implants (e.g., catheters), and even other bacterial cells.
- This adherence is crucial for colonization and the initiation of infection.
- Example: *Streptococcus mutans* (forms dental plaque).

c. **Protection Against Desiccation (Drying Out):**

- Due to its high water content, the capsule acts as a barrier, preventing the cell from drying out in dry environments. This enhances the survival of bacteria outside a host.

d. **Protection Against Toxic Substances:**

- The capsule can serve as a barrier, offering some protection against harmful chemicals, detergents, and heavy metals.

e. **Nutrient Reserve:**

- In some cases, the capsule can serve as a reserve of nutrients, which can be metabolized by the bacterium under starvation conditions.

f. **Protection Against Bacteriophages and Antibiotics:**

- The capsule can physically impede the access of bacteriophages to their receptors on the cell surface and may also offer some limited protection against certain antibiotics.

g. **Immunogenicity (Antigenic Properties):**

- Capsular polysaccharides can be highly antigenic and are often used as targets for vaccine development. For instance, the pneumococcal vaccine targets the capsular polysaccharides of *Streptococcus pneumoniae*.

(d) What is the significance of culture collections? Name any two microbial culture collection centres.

- **Significance of Culture Collections:**
  - Culture collections are vital repositories that collect, preserve, authenticate, and distribute diverse microbial strains (bacteria, fungi, archaea, algae, viruses, etc.). They play a crucial role in various scientific, industrial, and educational sectors.
  - **Biodiversity Preservation:** They act as living gene banks, preserving microbial diversity for current and future generations. This is critical for safeguarding potentially useful strains that might be lost from natural environments.
  - **Research and Development:** Provide authenticated and well-characterized strains for scientific research (e.g., genetics, physiology, ecology), drug discovery, enzyme production, and biotechnology. Researchers rely on these collections for reproducible experiments.
  - **Quality Control and Standardization:** Serve as sources for reference strains used in quality control for food, pharmaceutical, and other industries, ensuring product safety

and efficacy. They also provide type strains for taxonomic studies.

- **Education:** Offer educational resources and strains for teaching microbiology in academic institutions.
- **Intellectual Property:** Maintain strains related to patents and intellectual property, providing legal protection and access to patented biological material.
- **Public Health:** Provide strains for diagnostic testing, epidemiological studies, and vaccine development.
- **Bioprospecting:** Facilitate the search for new and useful microbial products (e.g., antibiotics, enzymes, biofuels) by providing access to diverse strains.

- **Name any two microbial culture collection centres:**

a. **ATCC (American Type Culture Collection):** Located in Manassas, Virginia, USA. It is one of the largest and most widely recognized global biological resource centers.

b. **NCIMB (National Collection of Industrial, Food and Marine Bacteria):** Located in Aberdeen, Scotland, UK. It specializes in bacteria of industrial, food, and marine importance. *(Other notable examples include DSMZ - German Collection of Microorganisms and Cell Cultures, RIKEN BRC - BioResource Research Center in Japan, etc.)*

Question 6: (a) Give reasons for the following : (3×4=12) (i) Agar is used as a solidifying agent in culture media. (ii) Streaking is a dilution process. (iii) Length of lag phase varies in different bacterial cultures. (iv) Archaea are insensitive to penicillin. (b) What are SASPs and what is their function? (3) (c) How is the composition of bacterial ribosome different from that of eukaryotic ribosome? (3)

(a) Give reasons for the following:

- **(i) Agar is used as a solidifying agent in culture media.**
    - **High Melting and Solidification Points:** Agar melts at approximately 95°C and solidifies at around 42°C. This wide temperature differential is crucial because it allows the molten agar to be mixed with heat-sensitive nutrients and bacterial inocula (which would be killed at 95°C) and then poured into Petri dishes without solidifying too quickly. Once solidified, it remains solid at typical incubation temperatures (e.g., 37°C) for bacterial growth.
    - **Non-Nutritive:** Agar itself is a complex polysaccharide derived from seaweed and is generally not metabolized (or very poorly metabolized) by most bacteria. This ensures that it acts purely as a solidifying agent without interfering with the nutritional requirements of the cultured microbes.
    - **Transparency:** Once solidified, agar is transparent, allowing for clear observation of microbial growth and colony morphology.
    - **Permeability:** It forms a firm gel that is permeable to water, nutrients, and small molecules, allowing them to diffuse through the medium and be accessible to the growing microbes.
    - **Stability:** It is stable over a wide range of pH values commonly used in microbiology.
- **(ii) Streaking is a dilution process.**
    - Streaking (specifically the quadrant streak or T-streak method) is considered a dilution process because its primary goal is to physically reduce the number of microbial cells in different sections of an agar plate, leading to the isolation of individual colonies.
    - **Mechanism:** When a bacterial sample is spread across the surface of an agar plate using a sterile loop, the initial inoculum in the first section (quadrant) is dense. As the loop is re-

sterilized and dragged from the first section into subsequent sections, fewer and fewer cells are transferred with each drag. This progressive reduction in cell density eventually leads to areas on the plate where individual cells are deposited far enough apart that they can grow into discrete, visible colonies, each originating from a single cell (or a cluster of identical cells).

- This dilution makes it possible to obtain pure cultures from a mixed population, as distinct colonies represent clonal populations.

- **(iii) Length of lag phase varies in different bacterial cultures.**
  - The length of the lag phase, the initial period after inoculation where there is no significant increase in cell numbers, varies due to several factors:
    - **Physiological State of Inoculum:** If the inoculum comes from a healthy, actively growing (log phase) culture of the same species and is transferred to the same medium, the lag phase will be short or even absent. If the inoculum comes from an old, stationary, or death phase culture, the cells may be metabolically stressed and damaged, requiring a longer time to repair and synthesize necessary components before growth can resume.
    - **Previous Growth Medium vs. New Medium:** If the bacteria are transferred from a rich medium to a chemically different or poorer medium, they need time to synthesize new enzymes and metabolic pathways required to utilize the available nutrients, leading to a longer lag phase. Conversely, transfer to a richer medium may shorten the lag phase.
    - **Temperature and pH Shock:** Abrupt changes in temperature or pH between the previous culture and the

new growth conditions can induce cellular stress, prolonging the lag phase as cells adjust and recover.

- **Inoculum Size:** A very small inoculum size might result in a slightly longer apparent lag phase simply because it takes more time for enough cells to divide to register a detectable increase in population.
- **Species-Specific Adaptations:** Different bacterial species inherently have different metabolic capabilities and adaptive responses to environmental changes, which influences their lag phase duration.

- **(iv) Archaea are insensitive to penicillin.**
  - Archaea are insensitive to penicillin because the primary target of penicillin and other beta-lactam antibiotics is **peptidoglycan**, a unique component of the bacterial cell wall. Penicillin inhibits the transpeptidase enzymes (penicillin-binding proteins or PBPs) responsible for cross-linking peptidoglycan strands, thus weakening the cell wall and leading to lysis.
  - **Archaea do not have peptidoglycan in their cell walls.** Instead, their cell walls are composed of diverse materials such as:
    - **Pseudomurein (Pseudopeptidoglycan):** In some archaea, this polymer structurally resembles peptidoglycan but has different chemical linkages (β-1,3 glycosidic bonds instead of β-1,4) and uses N-acetyltalosaminuronic acid instead of N-acetylmuramic acid. Critically, the peptide cross-links are made of L-amino acids, not the D-amino acids found in bacteria. These structural differences mean that archaeal cell wall synthesis enzymes are not inhibited by penicillin.
    - **S-layers:** Many archaea have S-layers as their outermost cell wall.

- **Polysaccharides or Glycoproteins:** Other archaea have cell walls composed of various polysaccharides or glycoproteins.

- Since Archaea lack the specific target (peptidoglycan) that penicillin acts upon, they are naturally resistant to this antibiotic.

(b) What are SASPs and what is their function?

- **SASPs (Small Acid-Soluble Spore Proteins):**
  - SASPs are a group of unique proteins found exclusively in the core of bacterial endospores. They are small, highly abundant, and characterized by their solubility in acid.
  - They are synthesized during sporulation (the process of endospore formation).
- **Function:**

a. **DNA Protection:** The primary and most critical function of SASPs is to protect the endospore's DNA from various forms of damage (e.g., UV radiation, desiccation, heat, chemicals).
   - They tightly bind to the DNA in the spore core, effectively saturating it. This binding changes the conformation of the DNA from the normal B-form to a more compact A-form, which is more resistant to pyrimidine dimer formation caused by UV radiation.
   - This tight binding also makes the DNA more resistant to chemical modification and enzymatic degradation.

b. **Carbon and Energy Source:** During germination, when the endospore begins to grow into a vegetative cell, SASPs are rapidly degraded by specific proteases. The amino acids released from this degradation serve as a vital source of carbon and energy for the synthesis of new proteins during the early

stages of germination, before external nutrients become available.

- In essence, SASPs are key to the extraordinary resistance of bacterial endospores and their ability to survive harsh conditions for extended periods.

(c) How is the composition of bacterial ribosome different from that of eukaryotic ribosome?

- Ribosomes are cellular organelles responsible for protein synthesis (translation). While both bacterial (prokaryotic) and eukaryotic ribosomes perform the same fundamental function, they differ significantly in their size, composition, and sensitivity to antibiotics.
- **Bacterial (Prokaryotic) Ribosome:**
  - **Size:** 70S (Svedberg unit, indicates sedimentation coefficient).
  - **Subunits:** Composed of two subunits:
    - **Large Subunit:** 50S subunit.
      - Composed of two ribosomal RNA (rRNA) molecules: 23S rRNA and 5S rRNA.
      - Contains approximately 34 different proteins.
    - **Small Subunit:** 30S subunit.
      - Composed of one rRNA molecule: 16S rRNA.
      - Contains approximately 21 different proteins.
  - **Location:** Free in the cytoplasm.
  - **Antibiotic Sensitivity:** Sensitive to many common antibiotics (e.g., streptomycin, tetracycline, chloramphenicol, erythromycin), which target specific differences between prokaryotic and eukaryotic ribosomes, thus selectively inhibiting bacterial protein synthesis without harming host cells.

- **Eukaryotic Ribosome:**
  - **Size:** 80S.
  - **Subunits:** Composed of two subunits:
    - **Large Subunit:** 60S subunit.
      - Composed of three rRNA molecules: 28S rRNA, 5.8S rRNA, and 5S rRNA.
      - Contains approximately 49 different proteins.
    - **Small Subunit:** 40S subunit.
      - Composed of one rRNA molecule: 18S rRNA.
      - Contains approximately 33 different proteins.
  - **Location:** Can be free in the cytoplasm or attached to the endoplasmic reticulum, or found in mitochondria and chloroplasts (which have 70S ribosomes).
  - **Antibiotic Sensitivity:** Generally insensitive to antibiotics that target 70S ribosomes.