

BIOCHEMISTRY OF MICROBES

Morphology, nutrition and physiology of bacteria

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Keywords

Bacteria; Peptidoglycan; Plasma membrane; Transport systems in bacteria; Endospores; Bacterial growth; Culture media.

Introduction

Bacteria are small prokaryotic cells with the size range usually varying from 0.5 to 1 μm in diameter and 1 to 6 μm in length. Lately, there are reports related to the existence of even much smaller (about 0.1 μm diameter) bacteria in nature, which are referred as nanobacteria. The smaller size of bacterial cell provides a definite advantage over other organisms with respect to the exchange of nutrients from its environment. Since, a smaller sphere has a higher surface area to volume (S/V) ratio as compared with a larger sphere, the exchange of nutrients will be more efficient in small cells. Thus, per unit of available nutrients, the small cells will typically yield a larger population than larger cells. For instance, spherical bacteria with a diameter of 2 μm , surface area of about 12 μm^2 and a volume of 4 μm^3 , will have the S/V ratio is 3:1. In contrast, a eukaryotic cell with a diameter of 20 μm has a surface area of about 1200 μm^2 and volume of 4000 μm^3 . Their S/V ratio is 0.3:1. The large S/V ratio of bacteria means that internal parts of the cell are very close to surface and, therefore, the nutrients can easily and rapidly reach all parts of the cell. Predominantly, the bacteria are observed in three common shapes: spherical, rods and spiral. These spherical bacteria are called cocci (singular coccus), whereas those shaped like a cylinder are termed as rods or bacilli. Some bacteria are shaped like a spiral and known as helicals. The spiral bacteria also exist in a variety of shapes. A comma-shaped bacterium is called a Vibrio. A rigid, wavy-shaped bacterium is spirillum; and corkscrew-shaped is spirochete. Some other bacteria have atypical morphology, like spindle-shaped, square-shaped (Haloarcula), and star-shaped (Stella). Moreover, those without a well-defined shape are referred as pleomorphic. Morphologically, the bacteria can be further classified based on their colony shape and size variability. They can also be categorized on the basis of their cell wall properties as: Gram-negative; Gram-positive; without a cell wall – the mycoplasmas, and cell wall lacking peptidoglycan – the archaea. The Gram-negative and Gram-positive bacteria are ubiquitous and widely prevalent in soil, water, air and sub-surface environment. However, the Archae bacteria are normally found in harsh environments, like acidic conditions (acidophiles); high salt (halophiles) and dry habitat (xerophiles).

General structure of bacterial cell

The bacterial cell (Fig. 1) is comprised of a cell envelope that mainly includes: cell membrane, cytoplasm, ribosomes, and a nucleoid. Most bacteria have a cell wall, although a few do not. Other constituents such as flagella or pili are commonly observed but not essentially present in all bacteria.

Cell envelope

The cell envelope is an illustrative term used to depict the multiple layers of biological material that surrounds or envelope the protoplasm of the cell. In other words, the cell protoplasm (cytoplasm) is protected by the plasma membrane, a cell wall and a capsule. The bacterial cell wall itself is a layered structure. It protects the underlying protoplast from all kinds of damage. Many bacteria have a polysaccharide capsule, or at least a glycocalyx present at the outer surface of the cell wall. The details of the cell envelope are discussed below.

Capsules

Most bacteria form a capsular polysaccharide layer external to the cell. A true capsule as a discrete detectable layer of polysaccharides deposited outside the cell wall is easily

detectable upon staining with Indian ink (Fig. 2). In some bacteria, a less discrete structure or matrix is formed, which embed the cells and is called a slime layer or a biofilm. The thin layer of tangled polysaccharide is also called as glycocalyx. The capsule exhibits several functions, including (i) adherence of cells to surfaces, (ii) protection of bacterial cells from engulfment by predatory protozoa or phagocytes, (iii) protection against the attack by antimicrobial agents of plant or animal origin, (iv) protection of cells from perennial effects of drying or desiccation and (v) acts as a reserve of carbohydrate for metabolism.

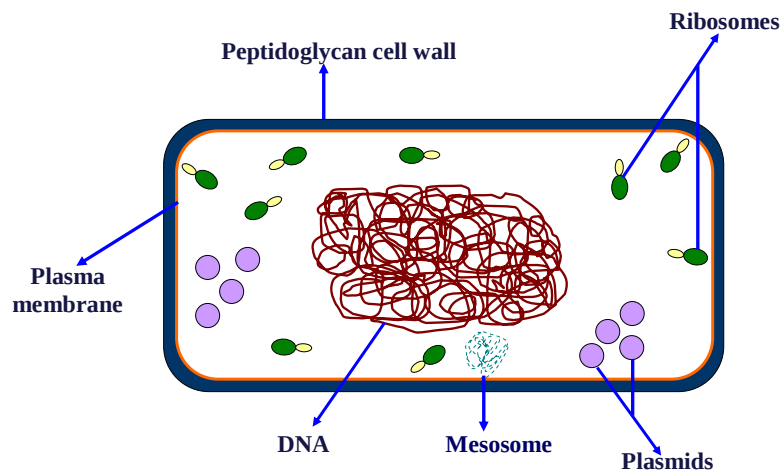


Fig. 1: Structure of a bacterial cell

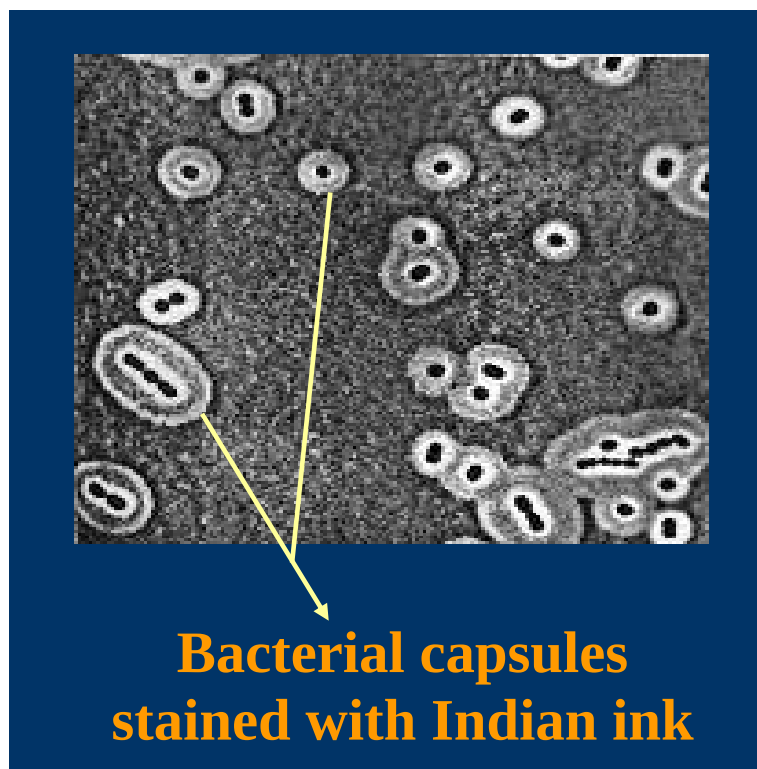


Fig. 2: Light microscopic view of bacterial capsule

Bacteria can attach to surface, produce slime, divide and produce microcolonies within the slime layer, and construct a biofilm, which provides an enriched and protected environment for their growth. A typical example of biofilm construction in nature is the formation of dental plaque mediated by the oral bacterium, *Streptococcus mutans*. The bacteria adhere specifically to the pellicle of the tooth by means of a protein on the cell surface. The bacteria grow and synthesize a dextran capsule which binds them to the enamel and forms a biofilm, some 300-500 cells in thickness. The bacteria are able to hydrolyze sucrose present in diet into glucose plus fructose. The fructose serves as an energy source for bacterial growth, whereas the glucose is polymerized into an extracellular dextran polymer that cements the bacteria to tooth enamel and becomes the matrix of dental plaque. The dextran slime on de-polymerization to glucose produces lactic acid within the biofilm or plaque that decalcifies the enamel and promotes dental caries.

Another important characteristic of capsule is the ability to block the phagocytic process and thereby prevent bacterial cells from being engulfed or destroyed by phagocytes. For example, the primary determinant of virulence of the pathogen *Streptococcus pneumoniae* is its polysaccharide capsule, which prevents ingestion of *Pneumococci* by alveolar macrophages. Similarly, the *Bacillus anthracis* survives phagocytic engulfment because the lysosomal enzymes of the phagocyte cannot initiate an attack on the poly-D-glutamate capsule of the bacterium. Bacteria such as *Pseudomonas aeruginosa* produce extracellular slime upon colonization and form a biofilm refractory to phagocytes.

Cell wall

The cell wall is an important structural component that protects the bacterial cell protoplast from mechanical damage and osmotic rupture. It is made up of a porous, rigid material that has high tensile strength, which can withstand the osmotic pressure equivalent to about 10-25 atmosphere against the inside of the plasma membrane. It also plays some role in determining the shape of the bacterial cell. The unique constituents of cell wall are not found elsewhere in nature. There are two types of cell walls found in bacteria. Based on the properties of their cell wall, the bacteria are categorized as Gram-positive and Gram-negative. Nevertheless, in both cases, the rigid protective nature of the wall is due to the macromolecule called as peptidoglycan or murein. This polymer is composed of alternating glycan molecules cross-linked by short peptides. The two glycans are *N*-acetylmuramic acid and *N*-acetylglucosamine. The Gram-positive cell wall consists of mostly the peptidoglycan plus acidic polysaccharides including teichoic acids. However, certain prokaryotes like the mycoplasma lack a cell wall, or the archaea with a modified cell wall (pseudomurein), do not contain peptidoglycan. The cell wall is an important bacterial cell component, which provides (i) an important site for attack by antibiotics, (ii) ligands for adherence and receptor sites for drugs or viruses, (iii) cause symptoms of disease in animals and (iv) immunological distinction and variation among strains of bacteria.

The structure of the cell walls of bacteria is shown in Fig. 3. In the Gram-positive bacteria the cell wall is thick (15 - 80 nm) and consists of several layers of peptidoglycan. It makes up about 40 to 80 % of dry weight of the wall, depending upon the species. It retains the purple crystal violet dye when subjected to the Gram-staining. On the contrary, the Gram-negative bacteria have a relatively thinner wall (10 nm), and contain a mono layer of murein, which does not allow the retention of crystal violet. They do not retain the primary dye, and appear pink-red when counter-stained with safranin dye (Fig. 4). In Gram-negative bacteria, the single layer of peptidoglycan is surrounded by a membranous structure called

the outer membrane. The outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS or endotoxin), which exhibits toxic effects. Chemically, the peptidoglycan in Gram-negative bacteria is made up of alternating molecules of *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) connected with a beta 1,4-glycosidic bond (Fig. 5). The 3-carbon of NAM is substituted with a lactyl ether group derived from pyruvate. The lactyl ether connects the glycan backbone to a peptide side chain that contains L-alanine, (L-ala), D-glutamate (D-glu), and D-alanine (D-ala). Strands of murein are assembled in the periplasm from about 10 muramic acid subunits. The strands are then connected to form a continuous glycan molecule that encompasses the cell. The tetrapeptide chains that project from the glycan backbone can be cross-linked by an interpeptide bond between L-Lys and D-ala aminoacids.

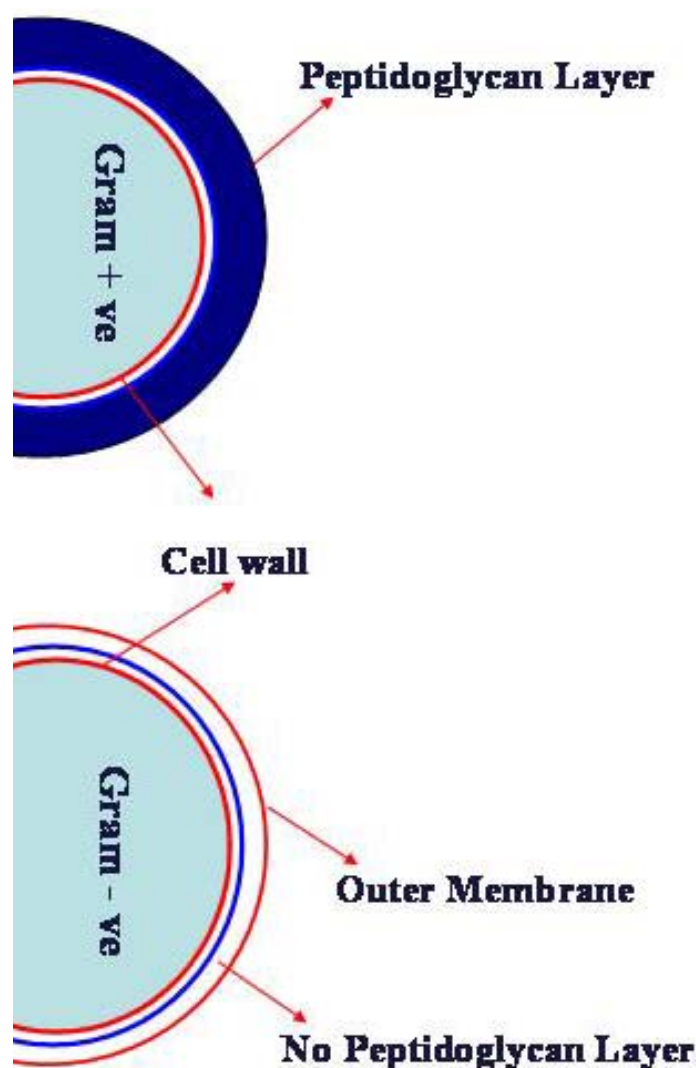


Fig. 3: Comparison of Gram + ve and Gram –ve bacterial cell wall

The assembly of peptidoglycan on the outside of the plasma membrane is mediated by a group of periplasmic enzymes, which are transglycosylases, transpeptidases and carboxypeptidases. It has been a general concept for several years that peptidoglycan

synthesis in cell wall defines the cell morphology. However, it is now clear that specific actin-like proteins exist in bacteria that define cell shape and that the peptidoglycan plays only a minor role. The major shape determining protein is MreB. The function of this protein is to form an actin-like cytoskeleton in bacteria and archaea. The protein MreB forms into filamental spiral bands around the cell just underneath the cytoplasmic membrane. The spherical (cocci) bacteria do not have MreB protein, which suggests that sphere is a 'default shape' for a bacterium.

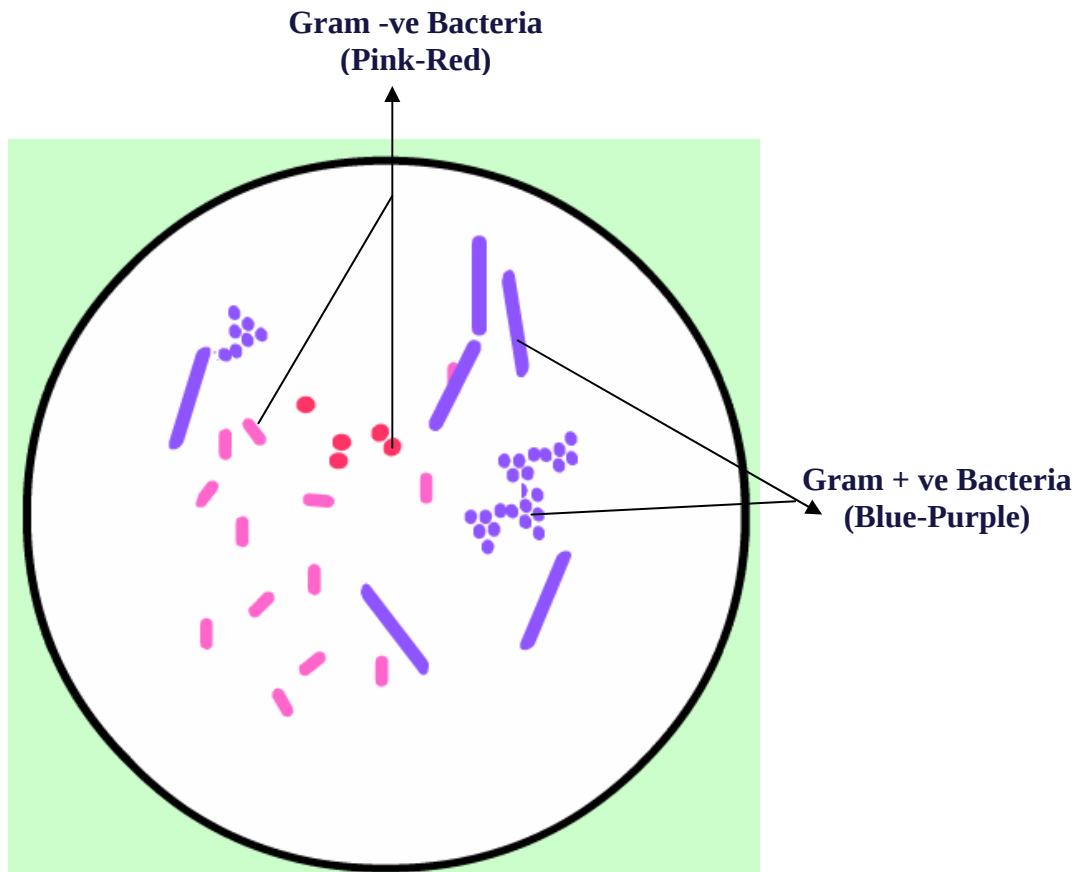


Fig. 4: Gram staining of bacterial cells

The Gram-negative bacteria are less vulnerable to attack by lysozyme because their peptidoglycan is shielded by the outer membrane. The exact site of lysozyme attack is the beta 1,4 bond between (NAM) and (NAG) on bacterial peptidoglycan. The peptidoglycans in Gram-positive bacteria exhibit several different peptide arrangements. Considerable variation exists in the amino acids that form the cross-linking peptides of peptidoglycan. Certain amino acids are never found in peptide bridge including sulfur-containing amino acids, aromatic amino acids and branched-chain amino acids, as well as arginine, proline and histidine. Since the peptidoglycan is not protected by an outer membrane, the Gram-positive bacteria are more sensitive to penicillin than Gram-negative bacteria. Some Gram-positive bacteria are also very sensitive to lysozyme. The glycan backbone of the peptidoglycan molecule is susceptible to cleavage by this enzyme present in animal serum, tissues and secretions, and in the phagocytic lysosome. The function of lysozyme is to lyse bacterial cells as a constitutive defense against bacterial pathogens. Closely associated with the layers of peptidoglycan in Gram-positive bacteria are a group of molecules called teichoic acids. Teichoic acids are linear polymers of polyglycerol or polyribitol substituted

with phosphates and a few amino acids and sugars. The teichoic acid polymers are often anchored to the plasma membrane called lipoteichoic acids apparently directed outward at right angles to the layers of peptidoglycan. The functions of teichoic acid are not very well understood. However, they are considered essential to viability of Gram-positive bacteria and provide a channel of regularly-oriented negative charges for threading positively charged substances through the complicated peptidoglycan network. It is also speculated that teichoic acids are in some way involved in the regulation and assembly of muramic acid subunits on the outside of the plasma membrane. Furthermore, in some specific cases, like in case of *Streptococci*, the teichoic acids are implicated in the adherence of the bacteria to tissue surfaces.

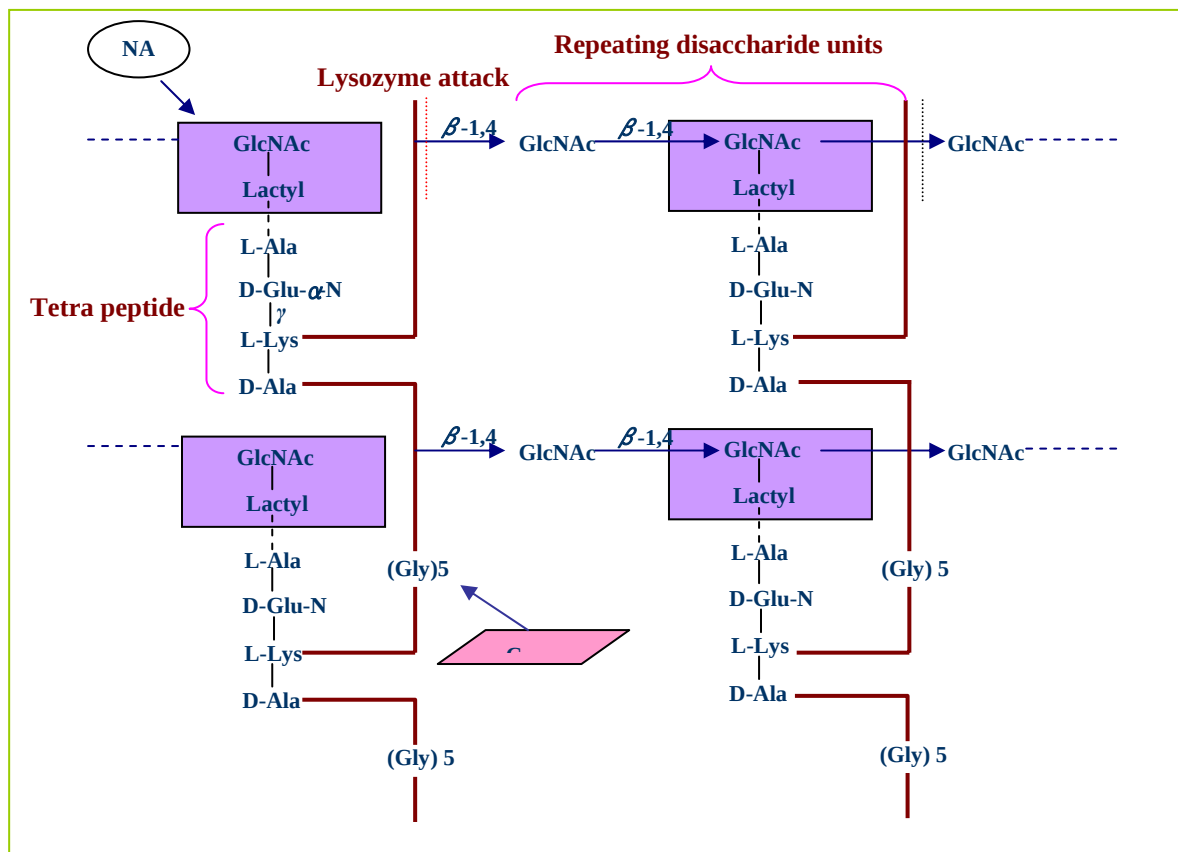


Fig. 5: A typical cell wall composition of Gram + ve bacteria

Cell wall-less forms

A group of bacteria called Mycoplasma exist without a cell wall. They have sterol-like molecules incorporated into their membranes and are usually inhabitants of osmotically-protected environments. Without a cell wall, they are polymorphic and mostly acquire the slender or branched filamentous growth. Some bacteria under pressure of antibiotic therapy lose their ability to form cell walls. These wall-deficient strains are called as L-forms, after the Lister Institute, where they were discovered. Moreover, some members of group Archaea may also lack cell wall.

Outer membrane of gram-negative bacteria

The outer membrane of Gram-negative cell wall is a discrete bilayered structure external to the peptidoglycan layer (Fig. 6). Indeed, it is the first and foremost permeability barrier. Owing to its lipopolysaccharide content, it possesses many interesting and important characteristics of Gram-negative bacteria. The outer membrane is a lipid bilayer intercalated with proteins, apparently similar to the plasma membrane. The internal side of the outer membrane is composed of phospholipids similar to the phosphoglycerides that compose the plasma membrane. The external face of the outer membrane may contain some phospholipids, but mainly it is formed by a different type of amphiphilic molecule, which is composed of lipopolysaccharide (LPS).

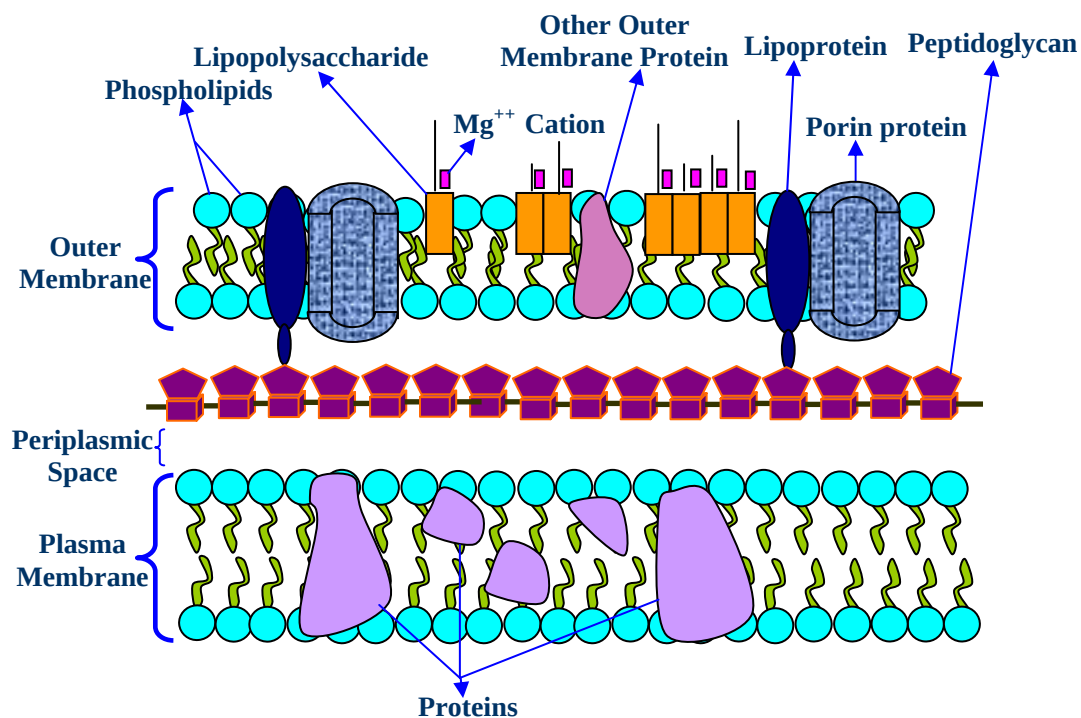


Fig. 6: Structure of Gram negative bacterial membrane and cell wall

The LPS molecule is composed of a hydrophobic region, called Lipid A that is attached to a hydrophilic linear polysaccharide region, consisting of the core polysaccharide and the O-specific polysaccharide (Fig. 7). The Lipid A molecule inserts into the interior of the membrane, and the polysaccharide part of the molecule is exposed to the aqueous environment. At the junction of lipid A and polysaccharide there is an accumulation of negative charge that provides the lateral stability to the outer membrane. The bacterial LPS are toxic to animals, and activate the (i) macrophages to produce pyrogens, (ii) complement cascade causing inflammation, and (iii) blood factors resulting in intravascular coagulation and hemorrhage, even when injected in small amounts. The toxic component of endotoxin (LPS) is Lipid A. The O-specific polysaccharide may provide ligands for bacterial attachment and confer some resistance to phagocytosis. Variation in the exact sugar content of the O-polysaccharide also referred to as the somatic or O-antigen, accounts for multiple antigenic types (serotypes) among Gram-negative bacterial pathogens.

and forms stack or vesicles attached to the inner membrane surface. These structures are called as mesosomes. Such internal membrane systems increase the surface area of membranes, like in cristae of mitochondria or the thylakoids of chloroplasts, to which enzymes are bound for specific functions. Mesosomes also represent the specialized membrane regions involved in DNA replication and segregation, cell wall synthesis, or increased enzymatic activity. Since bacteria do not have any intracellular organelles for respiration, photosynthesis or secretion, the plasma membrane is playing a role in accompanying these tasks for the cell. It performs a variety of functions related to energy generation, and biosynthesis. The electron transport system that couples aerobic respiration and ATP synthesis occurs in bacterial membrane. The light energy harvesting photosynthetic chromophores responsible for conversion of light into chemical energy are also localized in the membrane. Thus, the plasma membrane is the site of oxidative phosphorylation and photophosphorylation in bacteria, analogous to the functions of mitochondria and chloroplasts in eukaryotic cells. The bacterial membrane also contains the sensing proteins that measure concentrations of molecules in the environment and binding proteins that translocate signals to genetic and metabolic machinery in the cytoplasm. Membranes also contain enzymes involved in many metabolic processes such as cell wall synthesis, septum formation, membrane synthesis, DNA replication, CO₂ fixation and ammonia oxidation. An important function of plasma membrane is to provide a selective permeability barrier and to regulate the passage of substances into and out of the cell. The bacterial membrane freely allows the flow of water and uncharged molecules with MW of about 100 Daltons, and restricts the entry of larger molecules or any charged substances. Such restricted molecules are transferred only through the specialized membrane transport processes.

The specialized membrane-bound proteins that mediate the passage of solutes through cellular membranes are referred as carrier proteins, porters, and permeases. The transport systems operate by one of three transport processes: uniport, symport and antiport (Fig. 8). In a uniport process, a solute passes through the membrane unidirectionally. In symport or cotransport processes, the two solutes must be transported in the same direction at the same time; in antiport processes or exchange diffusion, one solute is transported in one direction and a second solute is transported in the opposite direction, simultaneously.

Transport systems in bacteria

Bacteria exhibit diverse types of transport systems for uptake of nutrients, which can be alternatively used in different environmental situations (Fig. 9). The evolution of transport systems in bacteria reflects their need to accumulate substances inside the cytoplasm against the concentration gradient of the environment. Concentration of solutes in the cytoplasm basically involves two types of active transport system; the ion-driven transport system and binding-protein dependent transport system. Furthermore, there are four types of carrier-mediated transport systems in bacteria. The carrier is a protein or group of proteins that functions in the passage of a small molecule across the membrane. A transport system can be a single transmembrane protein, which forms a channel for passage of a specific solute. It can also be a group of proteins, which sequentially bind and coordinate the passage of small molecule through the membrane. Most systems transport specific sugars, amino acids, anions or cations that have nutritional value to the bacteria. However, some transport systems exhibit the specificity for the solute to be transported.

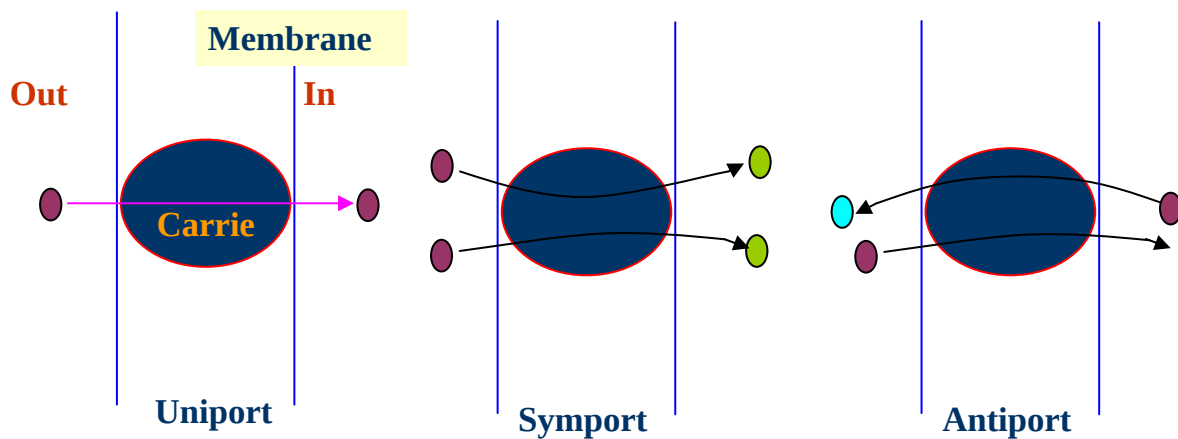


Fig. 8: Transport process in bacterial cells

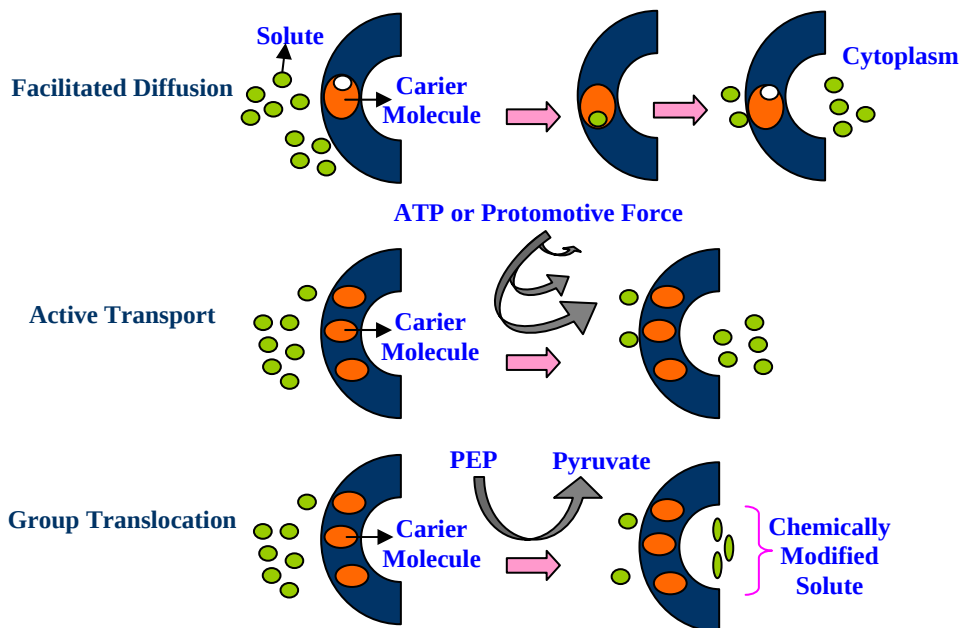


Fig. 9: Transport system in bacteria

Types of transport Systems in Bacteria

Facilitated diffusion: This is the least common type of transport system in bacteria. Indeed, the glycerol uniporter is the only well known facilitated diffusion system in bacteria. It involves the transport of a specific solute through a carrier that forms a channel in the membrane. The solute can move in either direction through the membrane to the point of equilibrium on both sides of the membrane. This system is specific and carrier-mediated, and does not require any energy for the transport process.

Ion driven transport systems: This is an active transport system, used for exchange of solutes, particularly ions and amino acids. It involves the symport or antiport process that uses a hydrogen ion (H^+) i.e., proton motive force (PMF), or chemiosmotic potential to

drive the transport process. For example, the lactose permease of *E. coli* utilizes the consumption of a hydrogen ion during the transport of lactose. The lactose permease is a single transmembrane polypeptide that spans the membrane seven times forming a channel and specifically admits lactose, requiring in the form of PMF.

Binding-protein dependent transport system: This is another active transport system, which helps in the transport of sugars and amino acids. For example, the histidine transport involves four proteins, out of which two proteins form a membrane channel that allows passage of histidine. The third protein resides in the periplasmic space where it is able to bind the amino acid and pass it to a fourth protein, which lets in the amino acid into the membrane channel. Hydrolysis of ATP provides the energy to drive the solute through the channel.

Group translocation system: This system also known as the phosphotransferase system, is primarily used for the transport of sugars in bacteria. For example, glucose transport involves group translocation process carried out by a phosphotransferase system. In this process a protein channel in membrane acts as a specific carrier for glucose. Glucose specifically passes through the channel from the outside and is phosphorylated to glucose-phosphate by the phosphotransferase system before it enters into the cytoplasm. The process derives energy from the intermediate phosphoenol pyruvate (PEP), which is hydrolyzed to pyruvate. Thus, with the consumption of high energy phosphate, one molecule of glucose is transported into the cell from external environment.

Flagella

Flagella are the filamentous protein structures attached to the cell surface. The diameter of a bacterial flagellum is about 20 nm, which is well below the resolving power of a light microscope. The ultrastructure of the flagellum of *E. coli* is illustrated in Fig. 10. Basically, the flagellar apparatus consists of a basal body, a hook-like structure and a flagellar filament. The structure involves several distinct proteins in the form of a system of rings embedded in the cell envelope. The inner most rings, the membrane (M) and super-membrane (S) rings, are associated with the plasma membrane, and comprise the motor apparatus. The periplasmic (P) and (L) rings, are located in the periplasm and the outer membrane, respectively, and function as bushings to support the rod where it is joined to the hook of the filament on the cell surface. As the M ring turns, powered by an influx of protons, the rotary motion is transferred to the filament, which turns to propel the bacterium. The flagellar filament is rotated by a motor apparatus in the plasma membrane allowing the cell to swim in fluid environments. Bacterial flagella are powered by proton motive force or chemiosmotic potential established on the bacterial membrane, rather than ATP hydrolysis, which powers eukaryotic flagella. About half of the rod-shaped (bacilli) and all of the spiral and curved bacteria are motile by means of flagella. However, very few cocci are motile, which reflects their adaptation to dry environments and lack of hydrodynamic design.

Flagella are distributed over the surface of bacterial cells in characteristic patterns. Flagellar arrangement with (i) a single flagellum (monotrichous) at one end is called as polar, (ii) multiple flagella emerging from one end is called lophotrichous, (iii) multiple flagella at both ends of the cell is called amphitrichous, and (iv) lateral flagella distributed over the entire cell surface is called peritrichous as shown in Fig. 11. The corkscrew-shaped bacteria called spirochetes have modified flagella known as axial filaments. These axial filaments

are also known as endoflagella because they are located within the cell envelope and impart a twisting motion to the cell.

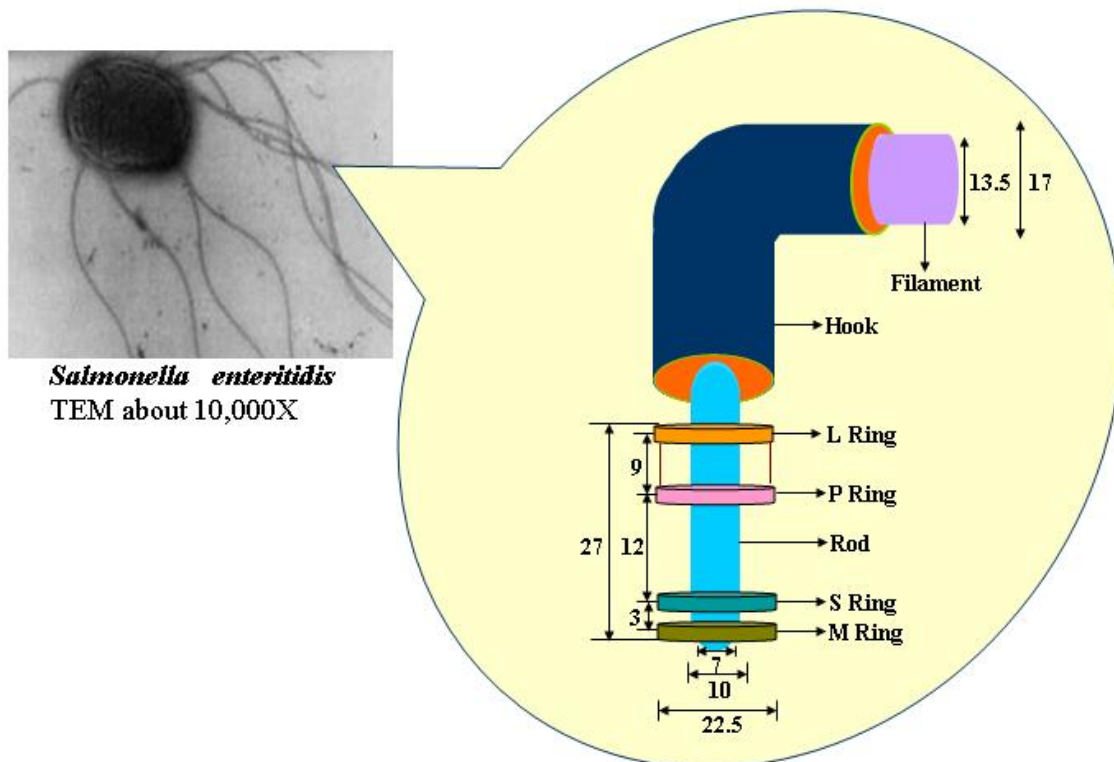


Fig. 10: Ultrastructure of a bacterial flagellum

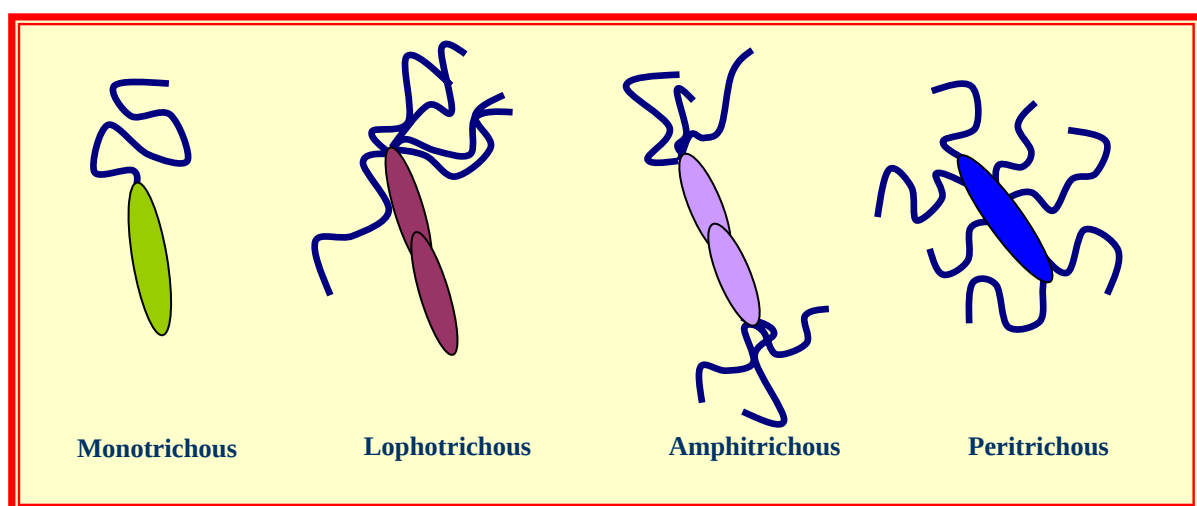


Fig. 11: Arrangements of bacterial flagella. Monotrichous: single polar flagellum; Lophotrichous: a cluster of polar flagella; Amphitrichous: flagella, either single or clusters, at both cell poles; Peritrichous: surrounded by lateral flagella

Flagella are the organelles of bacterial motility and if sheared, the cells can no longer swim. The flagella can regrow and as they reach a critical length, the swimming movement of cells is restored. The flagellar filament grows at its tip by the deposition of new protein subunits and not at its base.

Bacteria exhibit a variety of tactic behaviour (the ability to move or swim) in response to environmental stimuli. During chemotaxis, a bacterium can sense the quality and quantity of certain chemicals in its environment. They swim towards the chemicals, if they are useful nutrients or turn away from them, if they are harmful substances. Other types of tactic response in prokaryotes include phototaxis (movement in response to light), aerotaxis (movement in response to air-flow), and magnetotaxis (movement in response to magnetic field). The motility is regarded as a primary criterion for the diagnosis and identification of bacteria.

Fimbriae

Fimbriae (singular fimbria) or pili (singular pilus) are interchangeable terms used to designate short, hair-like structures on the surfaces of bacterial cells. They are shorter (about 1 μm in length and 3 to 5 nm in diameter), stiffer than flagella, and composed of protein (Fig. 12). Fimbriae are very common in Gram-negative bacteria, but occur in some archaea and Gram-positive bacteria. Fimbriae are responsible in adherence of bacteria to surfaces, substrates and other cells or tissues in nature. In clinical situations, they are major determinants of bacterial virulence because they allow pathogens to attach to tissues and/or to resist attack by phagocytes. For example, the pathogenic bacteria *Neisseria gonorrhoeae* adheres specifically to the human cervical or urethral epithelium by means of its fimbriae. The M-protein and associated fimbriae of *Streptococcus pyogenes* are involved in adherence and to resistance to engulfment by phagocytes. They are also responsible for microbial colonization of solids and formation of biofilms. The enterotoxigenic strains of *E. coli* adhere to the mucosal epithelium of the intestine by means of specific fimbriae. In *E. coli*, a specialized type of pilus, the F or sex pilus, mediates the transfer of DNA between mating bacteria during the process of conjugation.

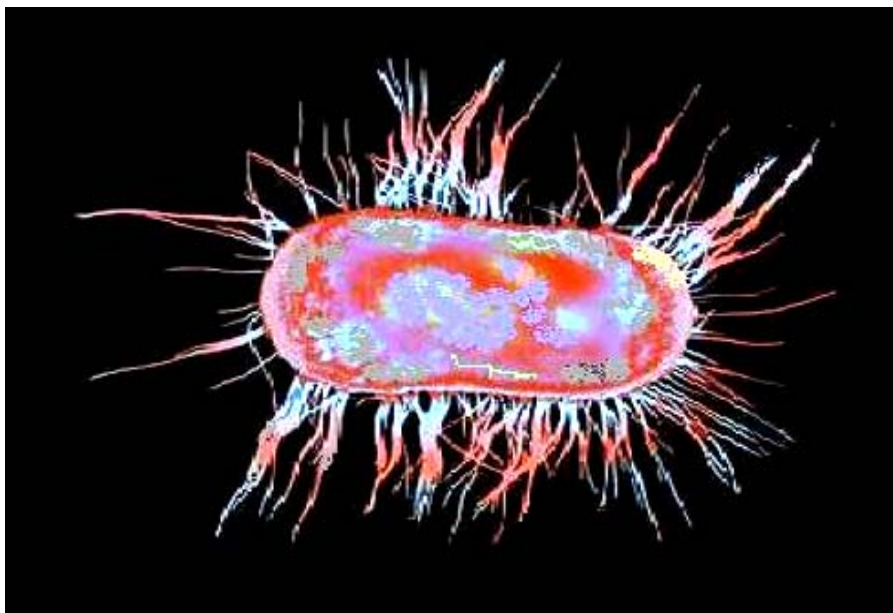


Fig. 12: Fimbriae (pili) on the surface of bacterial cell

Cytoplasm

The cytoplasm consists of metabolites and nutrients in solution. The chief constituents are water, salts, amino acids, and enzymes. Within the protoplasm are discrete entities known as the nucleoid, ribosomes, mesosomes, and granules. The bacterial cell does not have a true nucleus or a nuclear membrane. Instead, the DNA is folded into a dense material called the nucleoid. Bacteria sometimes also possess smaller extra-chromosomal pieces of DNA known as plasmids. The total DNA content of a cell is termed as genome. During cell growth and division, the bacterial genome is replicated in the usual semi-conservative fashion before distribution to progeny cells. Replication and segregation of bacterial DNA is coordinated by the membrane invaginations called mesosomes. The distinct granular appearance of bacterial cytoplasm is due to the presence and distribution of ribosomes. Ribosomes are particles responsible for protein synthesis. They are composed of a special type of RNA called ribosomal RNA (60%) and protein (40%). Bacterial ribosomes have a function similar to that of eukaryotic ribosomes but they significantly differ in structure. This difference is expressed in terms of sedimentation constants. The bacterial ribosomes are 70S units that contain subunits of 50S and 30S, whereas eukaryotic ribosomes are 80S units with 40S and 60S subunits. Ribosomes are also important in the identification of bacteria, as the nucleic acid sequences that code for the ribosomal RNAs are often unique to the bacterial cell type and used for phylogenetic analysis.

Inclusions

Bacteria have the ability to store the food reserves intracellularly as inclusions or granules. In cytoplasm of bacterial cells different types of inclusion granules are present, which occupy a substantial part of the cytoplasm (Table 1). For instance, the carbon and energy reserves are stored as glycogen or poly-beta-hydroxybutyric acid granules. Phosphate is stored in polyphosphate or volutin granules. Elemental sulphur is stored in sulphur globules by some phototrophic and lithotrophic bacteria. Some inclusion bodies are actually the membranous vesicles or intrusions into the cytoplasm, which contain photosynthetic pigments or enzymes.

Table 1: Types of inclusions in bacterial cells

Inclusions	Composition	Function
Carboxysomes	Autotrophic CO ₂ fixation	Site of CO ₂ fixation
Chlorosomes	Lipid and protein	Light-harvesting pigments
Gas vesicles	Protein hulls or shells inflated with gases	Buoyancy (floatation) in vertical water column
Glycogen	Polyglucose	Reserve carbon and energy source
Magnetosomes	Magnetite (iron oxide) Fe ₃ O ₄	Orienting and migrating along geo-magnetic fields lines
Poly-beta-hydroxybutyric acid (PHB)	Polymerized hydroxyl butyrate	Reserve carbon and energy source
Polyphosphate (volutin granules)	Linear or cyclical polymers of PO ₄	Reserve phosphate
Sulphur globules	Elemental sulphur	Reserve of electrons (reducing source) in phototrophs; reserve energy source in lithotrophs

Most bacteria do not form intracellular organelles bounded by unit membrane; the only possible exceptions are the thylakoids found in cyanobacteria. However, some bacterial organelles are bounded by non-unit membranes made up of proteins.

Gas Vacuoles

Most cells have a slightly higher density than water, and therefore, they tend to sink in the aqueous medium. This effect can be counteracted by swimming against the gravitational pull. The cells of aquatic bacteria contain gas filled structures known as gas vacuoles, which confer buoyancy on cells by decreasing their density. The gas vacuoles are compound organelles made up of variable number of gas vesicles. They are actually responsible for cells to float up and down in medium, in response to environmental factors. Gas vacuoles are present in certain purple and green phototrophic bacteria and some non-phototrophic bacteria that live in lakes and ponds. Also, the cyanobacteria and some species of archaea contain gas vacuoles. Gas vesicles in different organisms may vary in number from a few to hundreds per cell and in length from about 100 to over 300 nm and in width from 45 to 120 nm. The gas vesicle membrane is about 2 nm thick and is composed of protein permeable to gases but impermeable to water and solutes.

Carboxysomes

A number of photosynthetic bacteria (cyanobacteria, purple bacteria) and chemoautotrophic bacteria (nitrifying bacteria) contain the structure called as carboxysome. The carboxysome is a polyhedral cell inclusion generated from Calvin-Benson cycle of CO₂ fixation. The inclusion is about 100 nm in diameter and is surrounded by thin, non-unit membrane consisting of a tightly packed crystalline array of ribulose-bisphosphate carboxylase molecules (Rubis-co), a key enzyme in the fixation of CO₂. It helps in maintaining the amount of Rubis-co in the cell to allow rapid CO₂ fixation without affecting the osmolarity of the cytoplasm.

Magnetosomes

Magnetosomes are intracellular particles of magnetite (Fe₃O₄), the iron mineral. They help develop a magnetic dipole, which allows bacteria to respond to magnetic field, like in case of *Magnetospirillum* and *Magnetotacticum*. Magnetosomes producing bacteria exhibit magnetotaxis, the process of orienting and migrating along earth's magnetic field lines. Magnetosomes are surrounded by a non-unit membrane containing phospholipids, proteins and glycoproteins. They vary in shape from square to rectangular to spike shaped in certain bacteria, forming into chains inside the cell. Mostly the morphology of magnetosomes appears to be species-specific. The major function of magnetosomes is unknown.

Chlorosomes

In photosynthetic bacteria like in green-sulfur bacteria, a part of the photosynthetic apparatus has a distinctive intracellular location in unique structures called chlorosomes. The chlorosomes contain bacteriochlorophylls and are bounded by a thin, non-unit membrane attached to cytoplasmic membrane in the periphery of the cell.

Endospores

Endospores are the resistant bodies produced by some bacteria under adverse environmental conditions during the process called sporulation. They are refractile to heat, freezing, radiations and chemicals. The spore may be oval, ellipsoid or spherical and may occupy a central, sub-terminal or terminal position. An endospore consists of a protoplast or core surrounded by an integument or envelope. The spore core (protoplast) contains the genomic DNA and components of protein synthesis machinery like ribosome, tRNA, enzymes and other accessory factors. The spore envelope consists of the inner membrane, cortex, outer membrane and the spore coat (Fig. 13). In some bacteria, an additional covering called the exosporium is present. The inner membrane is a unit membrane covering the spore cytoplasm and nuclear material. The cortex is a multi-laminated structure, which develops between the inner and outer membranes. It consists of many layers of peptidoglycan. Endospores are formed by nearly 20 genera of bacteria as intracellular structures, and are ultimately released as free endospores. During sporulation, the synthesis of many proteins involved in vegetative cell functions stops. This is accomplished by activation of a variety of endospore-specific genes including the *spo* and *ssp* genes, which encodes small acid soluble proteins (SASPs). Accumulation of dipicolinic acid and calcium ions is characteristic of endospore. The function of calcium dipicolinic acid complex is to reduce water availability within the endospore and thus promote dehydration. In addition, the complex intercalates in DNA and stabilizes it to heat denaturation. The spores exhibit no signs of life, and are referred as cryptobiotic. However, they retain viability indefinitely and under appropriate environmental conditions, the spores germinate back into vegetative cells. Thus, the endospore-formation is a mechanism of survival, rather than a mechanism of reproduction.

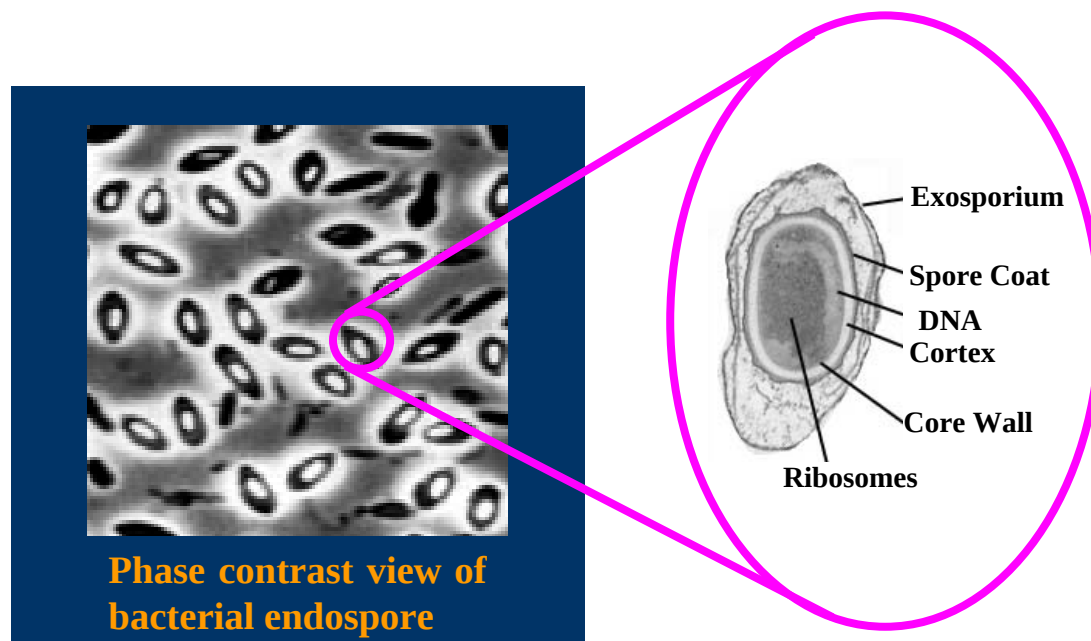


Fig. 13: Bacterial Endospore: Phase contrast view (A); Detailed structure of spore (B)

Bacterial nutrition

Bacteria require the necessary nutrients for growth and metabolism like any other life form. The vital compounds are water, protein, nucleotides, vitamins, minerals, etc., with about 97% of the dry weight of the cell being organic. Essential elements required in relatively large amounts are known as macronutrients and are generally involved in cellular metabolism. Elements that are required in smaller amounts are referred to as the micronutrients or trace elements. Micronutrients are often metals that are needed as catalysts for enzymes. Bacteria contain about 5000 different compounds; however, the number that most bacteria need to take up from the environment to synthesize these compounds can be as low as eight. For instance, *E. coli* require only mineral salts and glucose for survival, whereas other bacteria have requirements for preformed vitamins and other growth factors. The growth factors are organic molecules that are necessary for growth, which the microbes are unable to synthesize, for example, the vitamins, amino acids and nucleotides. The vitamins most commonly required by microorganisms are thiamine (vitamin B1), biotin, pyridoxine (vitamin B6), and cobalamin (vitamin B12). These nutrients are the chemical substances used by the organism for biosynthesis and energy generation. Bacteria must acquire all these elements from their environment for growth. Most often trace elements serve as cofactors in just a few enzymes, with only a small number of atoms being necessary per cell. The assortment of micronutrients and trace elements required by microorganisms is dependent upon its metabolic life style and it varies from species to species. Some species can use just a few simple compounds as their sources for all macronutrients, micronutrients and trace elements and can synthesize all the complex molecules required for growth. Other microbes do not have that broad metabolic repertoire and their growth depends upon certain organic molecules that the cell can not synthesize. The growth factors like vitamins, which are non-protein components of many enzymes, amino acids for protein synthesis, and nucleotides for DNA and RNA synthesis, are required exogenously. Certain bacteria do not require any organic nutrient for growth and are capable of living in nutrient-deficient environments; such bacteria are called as Oligotrophs. On the contrary, there are bacteria, which grow only in nutrient-rich environments, and such bacteria are called as copiotrophic bacteria.

Nutritional classification of microorganisms

Autotrophic bacteria are those that derive energy and carbon from inorganic sources and carbon dioxide. Autotrophs include both the photosynthetic and chemosynthetic bacteria. Photoautotrophs are photosynthetic and obtain energy from sunlight. Photosynthetic bacteria such as the cyanobacteria are important in causing eutrophication of surface waters. However, their prevalence in soil and vadose zones is limited because of light impermeability. Chemoautotrophs obtain energy by the oxidation of inorganic substances. Autotrophs are important in the cycling of inorganic nutrients, including the methanogens, producing methane gas and the nitrifiers converting ammonia to nitrate.

Heterotrophic bacteria are those that derive carbon from preformed organic compounds that are broken down enzymatically. The chemoheterotrophs derive energy through the oxidation of organic compounds via respiration. There are literally thousands of different types of chemoheterotrophs in the environment, and they are critical to many aspects of environmental microbiology including biogeochemical cycling. In addition, most pathogenic organisms are chemoheterotrophic. A few microbes, such as the green and purple sulphur bacteria, metabolise in a photoheterotrophic mode. These organisms get energy from light and use organic compounds as a source of reducing power. Generation of

energy through chemical oxidation is referred to as respiration regardless of whether the substrate is inorganic or organic. During the oxidative process, electrons are removed from the substrate and passed via the electron transport chain to a terminal electron acceptor. For aerobic bacteria the terminal electron acceptor is oxygen. However, for anaerobic bacteria, the terminal electron acceptor is a combined form of oxygen such as an organic metabolite CO_2 , NO_3^- or SO_4^{2-} or an oxidized metal, e.g., Fe^{3+} . Strict anaerobes lack protective enzymes for removing peroxide radicals that originate from O_2 metabolism. Other anaerobes do have the protective enzymes but still cannot use O_2 as a terminal electron acceptor. Some bacteria are facultative anaerobes, and they preferentially use O_2 , if it is present, and can also use other terminal electron acceptors, in the absence of O_2 . Facultative anaerobes can grow more efficiently in the presence of oxygen. The soil and vadose zone are always discontinuous environments, and thus different levels of oxygen availability (redox potential) may occur in close proximity to one another. This results in aerobic and anaerobic microbes being able to function in the same vicinity, which has important implications in areas of environmental microbiology including biodegradation of xenobiotics. Bacteria are extremely diverse with respect to their ability to transform organic and inorganic compounds under a variety of redox potential conditions.

Bacterial growth

Bacterial growth is defined as the increase in numbers of a microbial population. Division of a bacterial cell can occur either by binary fission, budding or filamentous growth. In all cases, the amount of DNA and the overall total mass of the bacteria increases over time. In other words, the term bacterial growth is an orderly increase in the quantity of cellular constituents. It depends upon the ability of the cell to form new protoplasm from nutrients available in the environment. In most bacteria, growth involves increase in cell mass and number of ribosomes, duplication of the bacterial chromosome, synthesis of new cell wall and plasma membrane, partitioning of the two chromosomes, septum formation, and cell division. This asexual process of reproduction is called binary fission. **Binary fission:** It occurs when an expanding cell splits and makes two cells. The division can be longitudinal, transverse, symmetrical or asymmetrical, it all depends upon the species. These cells then expand in size and, if conditions are favourable, further rounds of binary fission take place. Prior to division an indented ring in the cell wall and membrane, called a septum, forms around the middle of the cell's length. As the time of division grows nearer, the septum contracts eventually, pinching off and separating the two daughter cells. During this process the cytoplasm is divided and the newly replicated chromosomes are partitioned into separate daughter cells.

Budding: It is an alternative method of cell division in which a small protrusion expands outward from a mother cell, forming a daughter cell. The daughter cell increases in size until it breaks off from the mother cell. Eventually the daughter cell will reach a threshold size, and start producing buds of its own. This type of division is common in yeast cells and in certain strains of bacteria, especially stalked bacteria such as *Caulobacter*. During budding, a copy of the chromosome from the mother cell must pass into the daughter cell before the bud breaks off. Budding at the molecular level is almost same as the binary fission.

Filamentous growth: Some spore-forming bacteria like actinomycetes also exhibit filamentous growth and have an appearance similar to fungi. These microbes lengthen into a tubular shape as a long filament. At certain intervals the tube also branches in some species

and eventually expands in a number of directions. As actinomycetes grow, the copies of the genome are made and laid down along the filament. This allows a genome to always be available near the tip where growth is occurring. Many species produces cross walls that divide the filament into separate compartments, each with its own chromosome.

Bacterial growth measurement

Growth of bacteria can be measured in terms of changes in cell mass and in cell numbers. The cell mass can be measured by the direct and indirect methods. The direct method includes both the physical and chemical measurements. The physical measurements can be made in terms of dry-weight, wet-weight, or volume of cells, whereas the chemical measurements could be the total N, total protein, or total DNA contents. The indirect measurements include (i) measurement of chemical activity such as rate of O₂ production or consumption, CO₂ production or consumption, etc., and (ii) turbidity measurements. The turbidity or optical density of a suspension of cells is directly related to cell mass or cell number. The method is simple and non-destructive, but the sensitivity is limited to about 10⁷ cells per ml for most bacteria. The measurement of cell numbers involves direct counts and indirect viable cell counts.

Direct microscopic counts: The direct microscopic count has been used to observe and measure growth of bacteria in natural environments. It can be done by using special slides known as counting chambers or Petroff-Hausser chamber. Direct microscopic counting is a rapid way of estimating microbial cell number. However, it has several limitations such as (i) dead cells cannot be easily distinguished from living ones (ii) small cells are difficult to see under microscope, (iii) lacks precision, (iv) a phase contrast microscope is required to observe unstained specimen and difficulty in counting motile cells. Bacterial suspensions with cell density of >10⁷ cells per ml can be easily counted. If cells are in low density, they can be concentrated and then counted after staining with a fluorescent stain like fluorescein isothiocyanate (FITC) or 4, 6-diamino-2-phenylindole (DAPI)

Indirect viable cell count: It is also called as plate count or colony count, which involves the spreading of a serially diluted bacterial culture on a nutrient agar surface. On a suitable medium like nutrient agar, each viable unit grows and forms a colony. Each viable bacterium gives rise to a colony, which can be counted and is called a colony forming unit (cfu); and the number of cfu is related to the viable number of bacteria in the sample.

The advantages of this technique are the ease and sensitivity. The method also allows for inspection and identification of the bacteria grown on plates. The disadvantages are (i) only living cells develop colonies that are counted, (ii) clumps or chains of cells develop into a single colony, and (iii) colonies develop only from those organisms for which the cultural conditions are suitable for growth. The latter makes the technique virtually useless to characterize or count the total number of bacteria in complex microbial ecosystems such as soil or the animal gastrointestinal tract. For this purpose, the genetic probes can be used to demonstrate the diversity and relative abundance of bacteria. Many species identified by genetic techniques have so far proven to be unculturable.

Growth Cycle

Bacteria can be cultivated in a closed system (batch cultures), where nothing is added or removed during incubation or in an open system (continuous culture), where fresh medium

is added and the spent medium and cells are removed. Closed system consists of a single vessel, which may be a test tube, or a flask containing a definite amount of medium and are usually started from a small inoculum. The open system is more complex due to special requirements for aseptically adding and removing medium from the set-up called as a chemostat. The bacterial growth cycles in the batch and continuous cultures are discussed below:

Growth in Batch Cultures

When bacterial cells are grown in a batch culture, the rate of growth is not constant over time, as the conditions change in the flask. As the bacteria grow they utilize the nutrients in the flask and release the waste products in the medium. In case of the aerobic bacteria, as the population increases, the rate of oxygen consumption begins to exceed the rate at which it gets dissolved into the medium. Therefore, the growth rate slows down as the culture becomes oxygen-limited. The growth rate is defined as the number of doublings per unit time during the exponential phase. The geometric nature of the increase in cell number is a direct relationship between the number of cells present in a culture initially and the number after a known period of growth. This microbial activity causes a characteristic growth pattern that can be represented in four phases: lag phase, exponential or log phase, stationary phase and death phase (Fig. 14). Each of these phases reflects a distinct period of growth, and physiological changes occur due to shift from one phase to another.

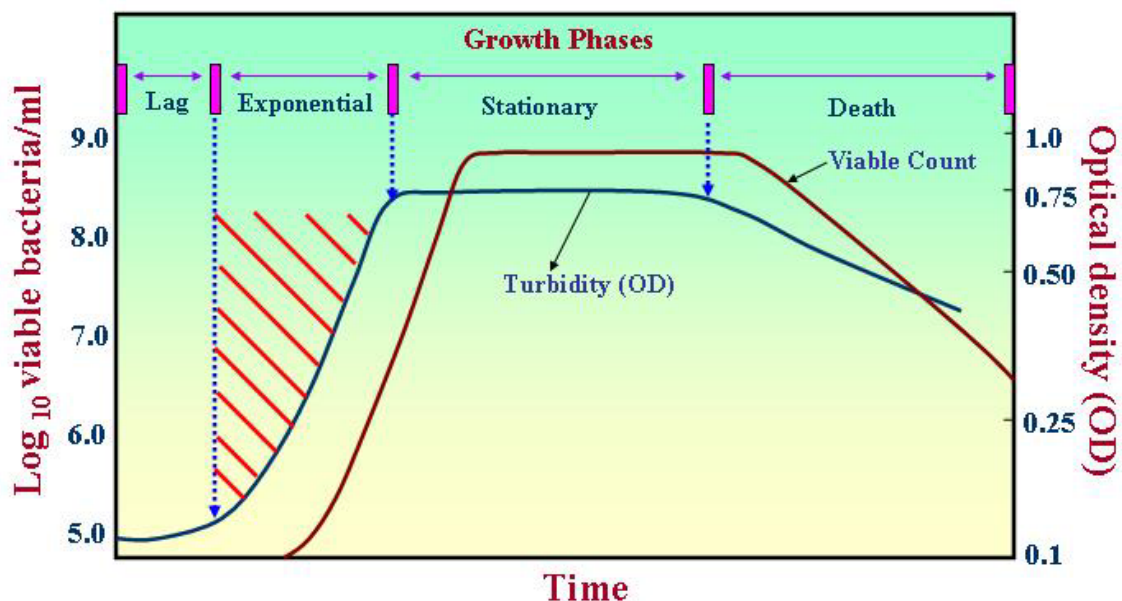


Fig. 14: Bacterial growth curves

The Lag phase

The first phase of growth curve under batch culture conditions is the lag phase. When an inoculum is added into fresh medium, the growth begins after a period of time called the lag phase. The lag phase is thought to be due to physiological adaptation of cell to the culture conditions. This may involve a time requirement for induction of specific RNA and protein synthesis to meet new culture requirements. The lag phase may also be due to low initial densities of organisms that result in dilution of exoenzymes (enzymes released from the

cell) and of nutrients that leak from growing cells. Normally, such materials are shared by cells in close proximity. But when cell density is low, these materials are diluted and not as easily taken up. As a result, initial generation time may be slowed until a sufficient cell density, approximately 10^6 cells/ml is reached. The lag phase usually lasts from minutes to several hours. The length of the lag phase can be controlled to some extent because it is dependent on the type of medium as well as on the initial inoculum size. For example, if an inoculum is taken from an exponential phase culture in Luria Broth (LB) and is placed into fresh LB medium at a concentration of 10^6 cells/ml, under the same growth conditions, there will be no noticeable lag phase. However, if the inoculum is taken from a stationary phase culture, there will be a lag phase as the stationary phase cells adjust to the new conditions and shift physiologically from stationary phase cells to exponential phase cells. Similarly, if the inoculum is placed into a medium other than LB, for example, a mineral salts medium with lactose as the sole carbon source, a relatively longer lag phase will be observed. This is because the cells will reorganize and shift physiologically to synthesize the appropriate enzymes for lactose catabolism. Furthermore, if the inoculum size is as small as 10^4 cells/ml, a lag phase will be observed until the population reaches to an optimal level of 10^6 cells/ml.

The Exponential phase

The next phase of growth observed in a batch system is the exponential or log phase. The exponential phase is characterized by a period of cell division when the rate of increase of cells in the culture is proportional to the number of cells present at any particular time. There are several ways in which this concept can be expressed. Consider that during exponential growth, the number of cells increases in the geometric progression $2^0, 2^1, 2^2, 2^4$ until, after n divisions at which the number of cells will be 2^n . Let us say, if the initial culture population is N_0 , then after growth for time t , the total bacterial population will be N_t , which can be determined from the initial population by using the following relationship.

$$N_t = N_0 2^n$$

Where, n is the number of generations.

$$\text{Thus, } \log_{10} N_t = \log_{10} N_0 + \log_{10} 2^n$$

$$n \log_{10} 2 = \log_{10} N_t - \log_{10} N_0$$

$$n = [\log_{10} N_t - \log_{10} N_0] / [\log_{10} 2]$$

$$n = 3.32 [\log_{10} N_t - \log_{10} N_0]$$

If the bacterial population in culture at two different time points is known, the number of generations from the initial population to the final population can be calculated. This formula only applies to actively growing cells in the exponential phase of growth. Also the growth rate (k) for any given culture can be determined by dividing the number of generations (n) with total time (t) as follows:

$$k = n/t = (3.32 [\log_{10} N_t - \log_{10} N_0]) / t$$

and the generation time can be determined as $t_{\text{gen}} = 1/k = t/n$.

The Stationary phase

The third phase of growth in a batch culture is the stationary phase. The stationary phase is defined as a state of no net growth, which can be expressed by the following equation:

$$dX/dt = 0$$

where, X is the number or mass of cells (mass / volume), and t is time.

Although there is no net growth in stationary phase, cells still grow and divide. Growth is simply balanced by an equal number of cells dying. The rationale is that the carbon and energy source or an essential nutrient becomes completely used up. When a carbon source is exhausted it does not necessarily mean that all growth stops. This is because dying cells can lyse and provide a source of nutrients. Growth on dead cells is called endogenous metabolism. Endogenous metabolism occurs throughout the growth cycle, but can be best observed during stationary phase when growth is measured in terms of oxygen uptake or evolution of carbon dioxide. Thus, in many growth curves, the stationary phase actually shows a small amount of growth. Again, this growth occurs after the substrate has been utilized and reflects the use of dead cells as a source of carbon and energy. A second reason that stationary phase may be observed is that waste products build up to a point where they begin to inhibit cell growth or exhibit cytotoxicity. This generally occurs only in cultures with high cell density. Regardless of the reason why cells enter stationary phase, growth in the stationary phase is unbalanced because it is easier for the cells to synthesize some components than others. As some essential components become more and more limiting, cells will still keep growing and dividing as long as possible. Therefore, due to this nutrient stress, the stationary phase cells are generally smaller and rounder than cells in the exponential phase.

The Death phase

The final phase of the growth curve is the death phase, which is characterized by a net loss of culturable cells. Even in the death phase there may be individual cells that are metabolizing and dividing, but more viable cells are lost as compared to the cells that are being produced, and therefore, there is a net loss of viable cells. The death phase is often exponential, although the rate of cell death is usually slower than the rate of growth during the exponential phase. The death phase can be described by the following equation:

$$dX/dt = -k_d X$$

where, k_d is the specific death rate.

It should be noted that the way in which cell growth is measured can influence the shape of the growth curve. If the growth is measured by optical density instead of the plate counts or CO_2 measurement, the onset of the death phase is not readily apparent. If conditions are not optimal, the bacteria grow more slowly, however division still occurs at an exponential rate, but not as rapidly as in the maximal growth rate. The highest rate of division is termed as the maximal growth rate. The effect of the environment on growth rate is difficult to generalize as the bacteria belonging to different phylogenetic groups have a unique set of optimal conditions. Besides, there are number of other environmental factors like temperature, pH, solute concentration, dissolved gases, nutrients and the presence of waste products, etc, which may influence the growth.

Growth in continuous culture

In a continuous culture, the bacteria are maintained in a state of exponential growth over long period of time. A glass fermentor called as a chemostat (Fig. 15) is used to maintain a bacterial population at a constant density, under conditions more or less similar to bacterial growth in natural environments. In a chemostat, the growth chamber is connected to a reservoir of sterile medium. Once growth is initiated, fresh medium is continuously supplied from the reservoir. The volume of medium in the growth chamber is maintained at a

constant level by regulated overflow to drain. Fresh medium is allowed to enter into the growth chamber at a rate that limits the growth of the bacteria. The bacteria grow at the rate at which the bacterial cells with spent medium are removed by the overflow. The rate of addition of the fresh medium determines the rate of growth because the fresh medium always contains a limiting amount of an essential nutrient. Thus, the chemostat relieves the insufficiency of nutrients, the accumulation of toxic substances, and the accumulation of excess cells in the culture, which are the parameters that initiate the stationary phase of the growth cycle. The bacterial culture can be grown and maintained at relatively constant conditions, depending on the flow rate of the nutrients.

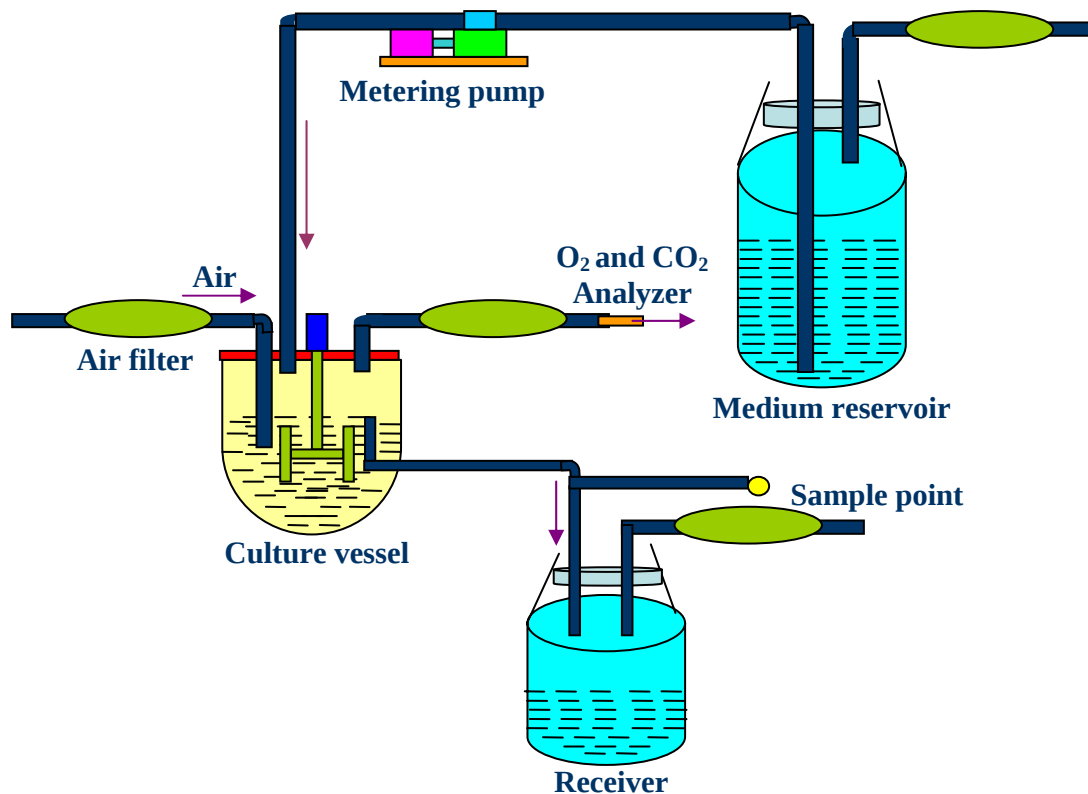


Fig. 15: Outline of a chemostat for continuous culture

Synchronous cultures

Synchronized growth of bacteria refers to the condition at which the bacterial cells in a growth medium are at the same stage of cell cycle. The growth of bacterial populations in batch or continuous cultures does not allow studying the growth behavior of individual cells, because of the random distribution of cell size among the members of the population. However, measurements made on synchronized cultures are equivalent to measurements made on an individual cell. Thus, study of synchronous culture provides information about the growth behaviour of individual bacteria. Bacterial populations at the same stage in the cell cycle can be obtained by manipulation of environmental parameters. One approach is to induce the population to start or stop the growth at the same point in the cell cycle, while others include physical methods like sieving for separating the new born cells with the aged cells.

Physical factors influencing the bacterial growth

Effect of Oxygen

Oxygen is an essential requirement for the life to sustain. However, bacteria display a wide range of responses to molecular oxygen. For instance, the obligate aerobes require O_2 for growth and use O_2 as a terminal electron acceptor in aerobic respiration. On the contrary, the obligate anaerobes (aerophobes) do not need or use O_2 as a nutrient. In fact, for anaerobes, the O_2 is a toxic substance, which either kills or inhibits their growth. Such kind of bacteria may live by fermentation, anaerobic respiration, bacterial photosynthesis, or by methanogenesis. Moreover, the facultative anaerobes can switch between aerobic and anaerobic types of metabolism. Under anaerobic conditions they grow by fermentation or anaerobic respiration, but in the presence of O_2 they switch to aerobic respiration. Aerotolerant anaerobes are bacteria with an exclusively anaerobic or fermentative type of metabolism but they are insensitive to the presence of O_2 . They live by fermentation alone whether or not O_2 is present in their environment.

The response of bacteria to O_2 in the environment depends upon the occurrence and distribution of various enzymes, which react with O_2 and various oxygen radicals that are invariably generated by cells in the presence of O_2 . For example, the oxidation of flavoproteins by O_2 results in the formation of H_2O_2 as a major toxic product and small quantities of even more toxic free radical, like superoxide anions ($O_2^{\cdot-}$). Also, the chlorophyll and other pigments in cells can react with O_2 in the presence of light and generate singlet oxygen, another radical form of oxygen which is a potent oxidant in biological systems.

In aerobes and aerotolerant anaerobes, the accumulation of superoxide is prevented by the enzyme superoxide dismutase. All bacteria, which can live in the presence of O_2 contain this enzyme. Most bacteria also contain the enzyme catalase, which decomposes H_2O_2 . Even though certain aerotolerant bacteria such as the lactic acid bacteria lack catalase, they decompose H_2O_2 by means of peroxidase enzymes, which derive electrons from $NADH_2$ to reduce peroxide to H_2O . Obligate anaerobes lack the superoxide dismutase and catalase and/or peroxidase, and therefore undergo oxidations upon exposure to O_2 . It is easy to determine the oxygen requirements of a microbe by simple laboratory procedures, one of the easiest being the thioglycollate test, which can categorize bacteria into different groups based upon their relationship to oxygen.

Reactions in thioglycollate

Thioglycollate agar contains thioglycollic acid, cysteine and agar that restrict oxygen to the top one-third of the tube. After inoculation and incubation, the pattern of growth in the tube determines the oxygen relationship with the test bacterial culture. In this medium, the aerobes grow only at the top of the tube. The facultative anaerobes grow throughout and strict anaerobes grow only at the bottom of the tube. Thus, thioglycollic acid and cysteine in agar restrict oxygen to the top one-third of the tube. The growth pattern in the tube determines the oxygen requirement and dependence.

There is an additional sub-class of microbes with a unique relationship with oxygen, the microaerophiles. These bacteria absolutely require oxygen as a nutrient, but cannot live in its presence at an atmospheric level. Microaerophiles are restricted to small niches where chemical gradients of oxygen exist that satisfy their needs, but at low enough levels that

they can survive. Microaerophiles have not been studied extensively due to problems in their cultivation. Many of the unculturable bacteria in the environment belong to this class.

Effect of temperature

Bacteria have a defined range of temperature within which they can grow optimally, and that reflects the environment in which they live. Those bacteria, which have a temperature optimum below 20°C and cannot grow above this temperature are termed psychrophiles. Bacteria with optima in the range of 20-45°C are mesophiles and those having temperature optima in the range of 45 to 48°C are called as thermophiles. Furthermore, the bacteria with temperature optima, above 80°C are called extreme thermophiles or hyperthermophiles. The psychrotrophs are a group of organisms that can grow slowly at low temperatures, but they normally have their optimum growth rate above 20°C, so they are also called mesotrophs. At low temperatures, growth is usually constrained by the fluidity of the membrane. The membrane begins to solidify, making it unable to carry out its many necessary functions. Bacteria overcome the low temperatures effect by synthesizing lipids containing fatty acids with double bonds. Some species isolated from the Antarctic region have polyunsaturated fatty acids, which are absent in other prokaryotes. Lipids with unsaturated fatty acids have lower freezing points and that maintains the membrane in quasi-fluid state even at lower temperatures. This is just like the vegetable oil, which contains polyunsaturated fatty acids and stays in liquid state even when refrigerated, while the butter consisting of saturated fatty acids solidifies. Low temperature is generally not lethal to bacterial populations. However, it reduces the rates of enzymatic reactions and at some critical level the reactions become so slow that growth ceases. Bacteria thriving at low temperatures also alter the structure of their enzymes so that they remain functional even at lower temperatures.

In thermophilic bacteria, the proteins form more compact structures. Thermal denaturation of protein is a problem and protein modifications are evident in thermophilic bacteria that live at high temperature. It is speculated that thermophilic enzymes are more rigid than their mesophilic homologues. Some thermophilic enzymes contain greater numbers of salt bridges and perhaps these ionic interactions play an important role in stability. Comparison of homologous proteins of mesophiles and thermophiles reveals that proteins from thermophiles have more hydrophobic amino acids with branched side chains. These may contribute to a more stable hydrophobic core for the thermophilic protein.

The effect of pH

Bacteria exhibit the ability to grow at a broad pH range. The range of pH over which the bacteria can thrive is defined by three cardinal points: the minimum pH, below which the organism cannot grow, the maximum pH, above which the organism cannot grow, and the optimum pH, at which the organism grows best. The bacteria with pH optima in the range of 1.0 - 5.5 are called as Acidophiles, for example, *Thiobacillus*, *Sulfolobus* and *Thermoplasma*. Neutrophiles grow optimally at a pH range of 5.5 to 8.0 and a majority of bacteria falls into this category. Pathogens of mammals usually have extraordinarily narrow pH ranges very close to 7.0 where they grow very well. Alkalophiles preferably grow at a pH range of 8.0 to 11.0. Such bacteria are found in highly basic soda lakes and high carbonate soils. Interestingly, the acidophiles and alkalophiles maintain their cytoplasm at a pH of around 7.0, irrespective of their environment. This is made possible by constantly pumping ions in and out of the cell to keep their internal pH neutral. If the pH of the cytoplasm is allowed to be acidic or alkaline, it would cause the denaturation of enzymes

and nucleic acid. Pumping the offending ions creates a proton motive force for the purposes of energy generation. For acidophiles this is clearly not a problem since there is an abundance of protons on the outside. In contrast, for alkalophiles, the concentration of protons inside the cell is actually higher. Thus, it is important to consider the optimum pH for growth of a desired bacterium and incorporate buffers in order to maintain the pH of the medium, which may change during growth due to accumulation of bacterial waste products.

The effect of water activity

Bacteria mostly require high water activity to be able to grow. Some natural environments such as salt lakes, soda lakes and deserts, etc., have low water activity. Hence the bacteria exhibit special molecular adaptations that allow them to grow in these environments. The problem of high salt is overcome by accumulating counter solutes in cell cytoplasm that balance the external osmotic pressure. These compatible solutes are proline, betaine, glycerol, trehalose, ribitol, sorbitol and mannitol, etc., which do not interfere with metabolism and are either pumped in from the environments by active transport or synthesized *de novo*. Halotolerant and moderate halophiles use amino acids and sugars as compatible solutes. Extreme halophiles pump in potassium ions and most of their enzymes only function in the presence of very high concentrations of potassium chloride. Below a threshold limit the bacteria are no longer able to transport the nutrient into the cell and this relates to the ability of the bacterial transport enzymes to bind it and move it inside. The affinities of these transport enzymes for their target nutrients and structural properties of the cell are one factor that determines where a microbe can exist in the environment. For instance, the *Caulobacter* species that produce long appendages called prothecae are typical example. The prothecae serve as a mode of attachment to surfaces, and also greatly increase the surface area of the cell. Consequently, this increases their ability to absorb nutrients. The prosthecae bacteria are adapted to living in very dilute environments and can survive in distilled water with the availability of trace nutrients.

Microbes that require high concentrations of salt in their environment are called halophiles. Mild halophiles require 1- 6 % salt, moderate halophiles require 6-15 % salt, and extreme halophiles require 15-30 % NaCl for growth. Halotolerant bacteria can endure moderate salt concentrations, but grow best in its absence. Many of the halophiles encounter NaCl or KCl in their natural environment and thus much of the halophiles use these salts as the solutes. Even though halophiles are osmophiles and halotolerant bacteria are osmotolerant, the term osmophiles is usually reserved for bacteria that are able to live in environments with high levels of sugar.

Culture media

Culture media are the nutrient solutions used to grow microorganisms in the laboratory. Because laboratory culture is required for the detailed study of bacteria, careful attention must be paid to both the selection and preparation of media for successful culture to grow.

Types of culture media

Two broad classes of culture media used in microbiology are: chemically defined media and undefined or complex media.

Chemically defined media

This type of media is prepared by mixing accurate amounts of highly purified organic and inorganic chemicals. Thus, the exact chemical composition of a defined medium is known. Since, all cells need large amounts of carbon to make new cell material the carbon is of great importance for any culture medium. In a simple defined medium usually a single carbon source is present. The extent and nature of carbon source depends on the type of bacteria to be cultivated.

Complex media

Complex media mainly consist of the extracts of animal or plant products such as casein, milk protein, beef, soybean, yeast cells, or any other highly nutritious substance. These digests are commercially available in powdered form and can be used quickly to prepare the medium. The major limitation in using a complex medium is that its precise nutrient composition is not known. However, for specific purpose, the culture media can be made selective or differential (or both). A selective medium contains the compounds that selectively inhibit the growth of some microorganism but not others. By contrast, a differential medium is one in which some sort of indicator dye is added that allows to differentiate the bacteria based on the colour of the colony. Differential media are quite useful for distinguishing between species of bacteria, some of which may carry out particular reactions while others do not contain any indicator dye that allows the differentiation between chemical reactions carried out during growth (Table 2). For example, Eosin-methylene blue (EMB) agar is widely used as a selective and differential medium. Eosin is a dye that responds to changes in pH and under acidic conditions undergoes a change from colourless to black. EMB agar is used for the isolation of Gram-negative enteric bacteria because this dye inhibits the growth of most Gram-positive bacteria. EMB agar contains lactose and sucrose but not glucose as energy sources. Thus, the lactose fermenting bacteria such as *Escherichia coli*, *Klebsiella*, and *Enterobacter* acidify the medium and colonies appear black with a greenish sheen. Colonies of non-lactose fermentors such as *Salmonella*, *Shigella*, and *Pseudomonas* are translucent or pink. Thus, EMB preferentially selects for the growth of Gram-negative bacteria, and also differentiates between several genera of the selected Gram-negative bacteria.

Solid and liquid culture media

Culture media can be prepared in semisolid or solid forms by the addition of agar (a sea weed) as a gelling agent in varying amounts to liquid medium. A solid medium allows bacteria to grow and form visible isolated mass of cells called colonies. Bacterial colonies can be of various shapes and sizes depending on the organism, the culture conditions, the nutrient supply (including the amount of oxygen present), and several other physiological parameters. Some bacteria produce pigments that make the colonies appear coloured. Generally, the microbiologists visualize the purity of the culture looking at the colonies. The solid medium that contains more than one type of colony is indicative of a contamination in the bacterial culture.

Table 2: Some common types of bacterial culture media

Types of media	Constituents	Uses
Chemically defined (synthetic) media: (e.g.)		
Ashbey's Agar Medium	Mannitol, MgSO ₄ , K ₂ HPO ₄ , CaCl ₂ , FeCl ₃ , NaCl, agar.	For cultivation of <i>Azotobacter</i> species.
Pseudomonas Isolation Agar	Peptic digest of animal tissue, K ₂ SO ₄ , MgCl ₂ , Triclosan (Irgasan), agar	For isolation of <i>Pseudomonas</i>
Complex or Chemically Undefined Media: (e.g)		
Czapek-Dox Agar Medium	NaNO ₃ , K ₂ HPO ₄ , MgSO ₄ , KCl, FeSO ₄ , Sucrose, agar	For general cultivation of fungi.
Malt extract Medium	Malt Extract, Mycological peptone, agar	For detection of Yeast and moulds
Rich media:		
Nutrient agar	Peptone, Beef Extract, NaCl, agar	General purpose culture medium
Selective media: (e.g.)		
Selective Lysine Agar	Peptic digest of animal tissue, Yeast extract, L-Lysine hydrochloride, Bile salts mix, Dextrose, Crystal violet, Bromocresol purple, Sulphapyridine, agar	For selective isolation of <i>Salmonella</i> and identification species
Mannitol salt agar medium	Proteose Peptone, Beef Extract, NaCl, D-Mannitol, Phenol red, agar	For isolation of pathogenic <i>Staphylococci</i>
Enrichment media: (e.g.)		
Selenite broth	Casein enzymatic hydrolysate, Lactose, Sodium hydrogen selenite, Na ₂ HPO ₄ , NaH ₂ PO ₄ , L-cystine, agar	For isolation of <i>Salmonella</i> from food, dairy products and pathological materials.
Enriched media: (e.g.)		
Blood agar	Beef heart Infusion, NaCl, Tryptose, 5 % v/v sterile defibrinated blood, agar	For cultivation of fastidious pathogenic bacteria such as <i>Neisseria</i> , <i>Streptococci</i> , etc.
Differential media: (e.g.)		
MacConkey agar	Peptone, NaCl, Lactose, Bile salt, Crystal violet and neutral red, agar	For selection and recovery of <i>Enterobacteriaceae</i> and related enteric Gram-negative <i>bacilli</i>

Suggested Reading

1. Black J. G. (2005) Microbiology Principles and Explorations (6th ed.) Wiley international Edition, USA.
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5. Perry J.J, Staley J.T, and Lory S. (2002) Microbial Life, Sinauer Associates Publishers. USA
6. Stanier R.Y, Ingraham J. L, Wheelers M.K, Painter P.R. (2005) General Microbiology, (5th ed.) Prentice-Hall, New Jersey, U.K