

# Bacteriology I

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# Chapter 1

## Cell Structure & Function

## Introduction

Bacteria were first observed by *Antony van Leeuwenhoek* in **1683**. The greatest pioneer in Bacteriology was the German **Robert Koch** who was the first to culture Bacteria. His earlier work was on the *Bacillus* responsible for *anthrax*.

The great French scientist, **Louis Pasteur**, disproved all beliefs concerning spontaneous generation and showed that bacteria are not spontaneously generated from putrefying material, but are spread about the earth either themselves or by means of spores. This immediately led to the reorganization of why diseases can be infectious, thus causing a healthy person to be attacked by a disease, without necessarily coming into actual contact with a diseased person. The disease is spread by means of the Bacteria floating in the air, or even more, by the lighter bacterial spores.

### **The Bacteria are characterized by the following features:**

- Have no true nucleus; genome is circular.
- No membrane-bound organelles.
- Cell wall usually contains peptidoglycan.
- Divide by binary fission.
- Some have storage granules.
- Some aquatic bacteria have gas vesicles.
- Some have endospores (soil bacteria) that enable them to lie dormant under “unfavorable” conditions.

### **I- Size of Bacterial Cells:**

The majority of bacteria measure approximately 0.5 to 1.0  $\mu$  (microns) by 2.0 – 5.0  $\mu$ . *Cocci* varies from about 0.2 to 4  $\mu$  in diameter,

*Bacilli* and *spirilla* usually resemble *cocci* in diameter but are longer cells of the *sulphur bacteria* are as much as 60 microns long and 25  $\mu$  thick. Some of the *spirochaetes* are even longer, up to 500 micron but still slender.

## II- The Occurrence of Bacteria:

Bacteria are very widely distributed, on and beneath the surface of the earth, in fresh water and in the sea, on and in other organisms, and on the dust particles which food is available to them. Almost all naturally occurring organic compounds can be used as food by one or another kind of bacteria, and some can derive energy through oxidation of inorganic substances.

Many Bacteria require free **oxygen**, others can use it or grow **anaerobically** without it, and others are **obligate anaerobes**. Some Bacteria live and grow at temperature as high as 75°C.

## Major Differences between Prokaryotes and Eukaryotes

	Prokaryotes	Eukaryotes
Chromosome structure	Relatively simple	Complex
Nucleus & Nuclear membrane	Not	Present
Meiosis and Mitosis	Not	Present
Cellulose and Chitin	Not	Present
Peptidoglycan	Present	Not
Mitochondria	Never present	Present
Chloroplasts	Never present	In photosynthetic Cells
Ribosomes	Only one type	Large type in Cytoplasm and Small type in Mitochondria
Flagella	When present has a simple structure	When present has a complex structure
Cell size	0.2-10 $\mu$ m in diameter	10-100 $\mu$ m in diameter

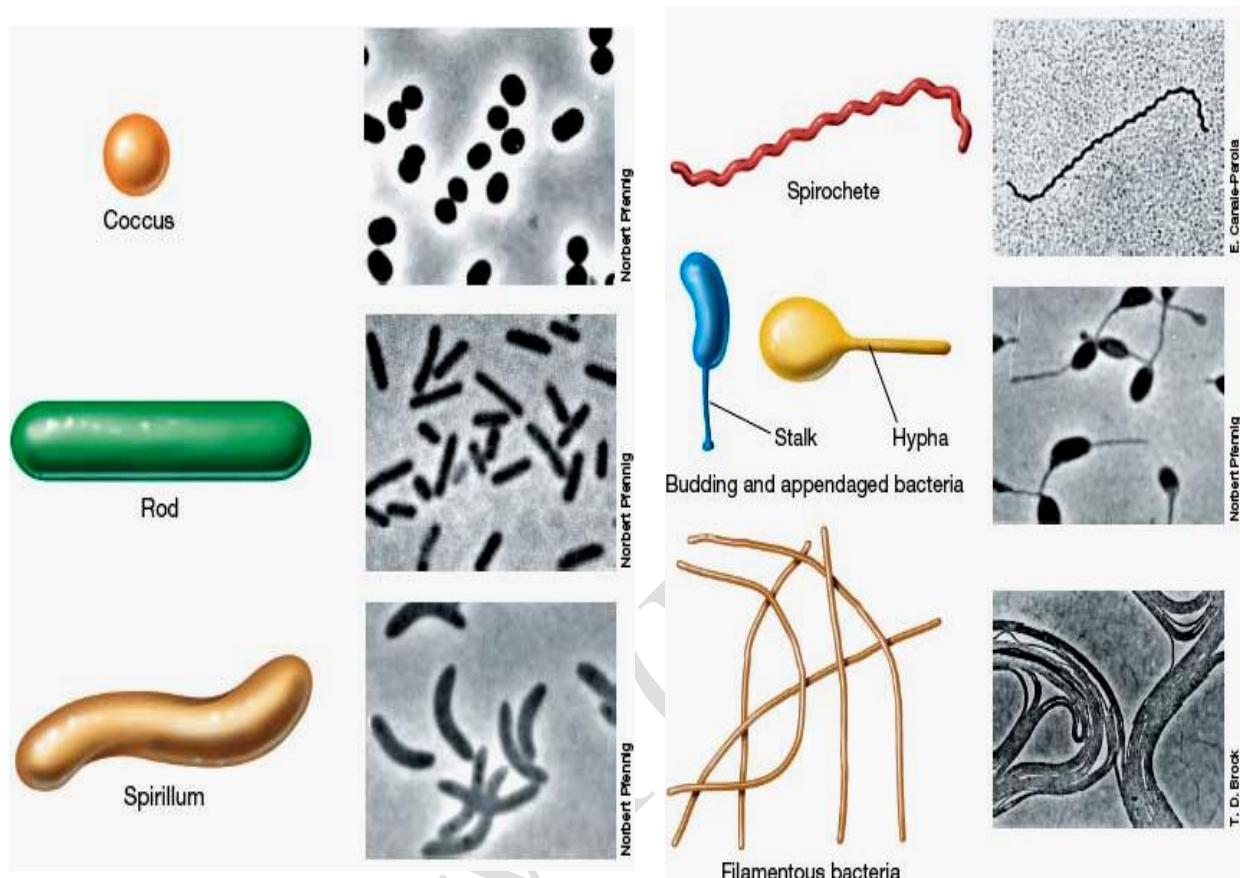
### **III- Morphology of Bacteria**

In microbiology, the term **morphology** means **cell shape**. Several morphologies are known among prokaryotes, and the most common ones are described by terms that are part of the essential lexicon of the microbiologist.

Bacteria display a wide diversity of shapes and sizes. Bacterial cells are about one tenth the sizes of eukaryotic cells and are typically 0.5–5.0 micrometres in length. Most bacterial species are either spherical, called cocci (*sing. coccus*), or rod-shaped, called bacilli (*sing. bacillus*). Elongation is associated with swimming. Some rod-shaped bacteria, called vibrio, are slightly curved or comma-shaped; others, can be spiral-shaped, called *spirilla*, or tightly coiled, called *spirochaetes*. A small number of species even have tetrahedral or cuboidal shapes. More recently, bacteria were discovered deep under the Earth's crust that grow as long rods with a star-shaped cross-section. The large surface area to volume ratio of this morphology may give these bacteria an advantage in nutrient-poor environments. This wide variety of shapes is determined by the bacterial cell wall and cytoskeleton, and is important because it can influence the ability of bacteria to acquire nutrients, attach to surfaces, swim through liquids and escape predators.

Many bacterial species exist simply as single cells, others associate in characteristic patterns: *Neisseria* form diploids (pairs), *Streptococcus* form chains, and *Staphylococcus* group together in "bunch of grapes" clusters. Bacteria can also be elongated to form filaments, for example the *Actinobacteria*. *Filamentous bacteria* are often surrounded by a sheath that contains many individual cells. Certain types, such as species

of the genus *Nocardia*, even form complex, branched filaments, similar in appearance to fungal mycelia.



Most bacteria come in one of the three basic shapes: **coccus, rod or bacillus, and spiral**.

### 1- The *coccus* shape:

The *cocci* are spherical or oval bacteria having one of several distinct arrangements based on their planes of division.

I. Division in **one plane** produces either a **diplococcus** or **streptococcus** arrangement.

**Diplococcus** is *cocci* arranged in pairs.

**Streptococcus** is *cocci* arranged in chains.

II. Division in **two planes** produces a **tetrad** arrangement.

a tetrad: *cocci* arranged in squares of 4

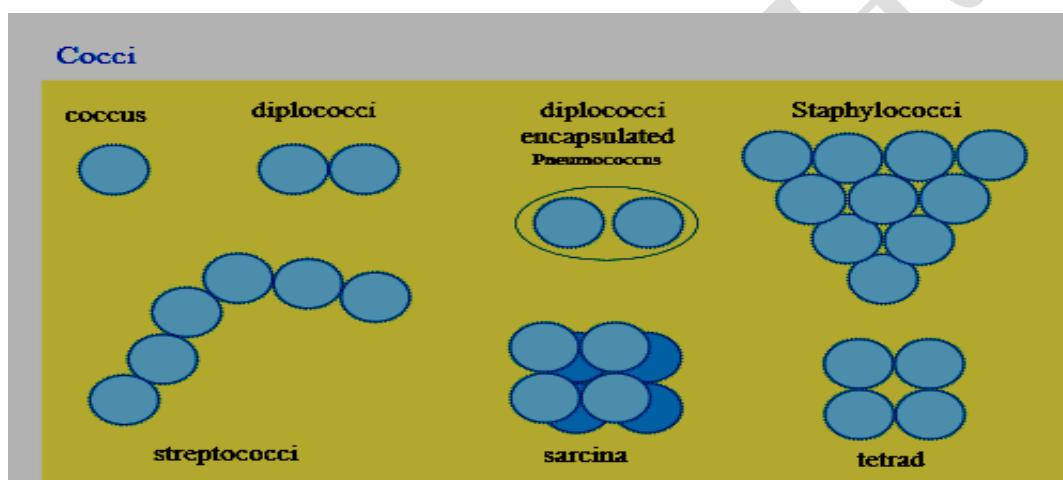
**III.** Division in **three planes** produces a *sarcina* arrangement.

*sarcina*: *cocci* in arranged cubes of 8

**IV.** Division in **random planes** produces a *staphylococcus* arrangement.

*staphylococcus*: *cocci* arranged in irregular, often grape-like clusters An average *coccus* is about 0.5-1.0 micrometer ( $\mu\text{m}$ ) in diameter. (A micrometer equals 1/1,000,000 of a meter.)

### Arrangements of *Cocci*



### 2. The rod or *bacillus* shape:

*Bacilli* are rod-shaped bacteria. *Bacilli* all divide in one plane producing a *bacillus*, *streptobacillus*, or *coccobacillus* arrangement.

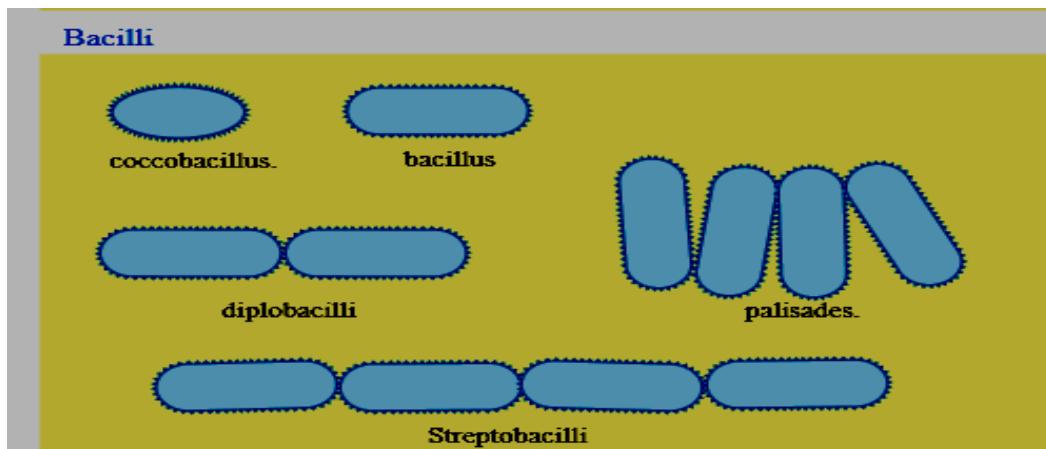
a. *Bacillus*: single *bacilli*

b. *Streptobacillus*: *bacilli* arranged in chains

c. *Coccobacillus*: oval and similar to a *coccus*

An average *bacillus* is 0.5-1.0  $\mu\text{m}$  wide by 1.0 - 4.0  $\mu\text{m}$  long.

### Arrangements of *Bacilli*

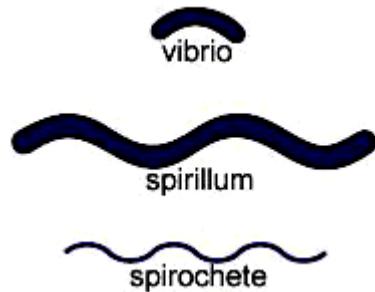


### 3. The spiral shape:

Spirals come in one of three [forms](#), a *vibrio*, a *spirillum*, or a *spirochete*.

- a. *vibrio*: a curved or comma-shaped rod
- b. *spirillum*: a thick, rigid spiral
- c. *spirochete*: a thin, flexible spiral Spirals range in size from 1  $\mu\text{m}$  to over 100  $\mu\text{m}$  in length.

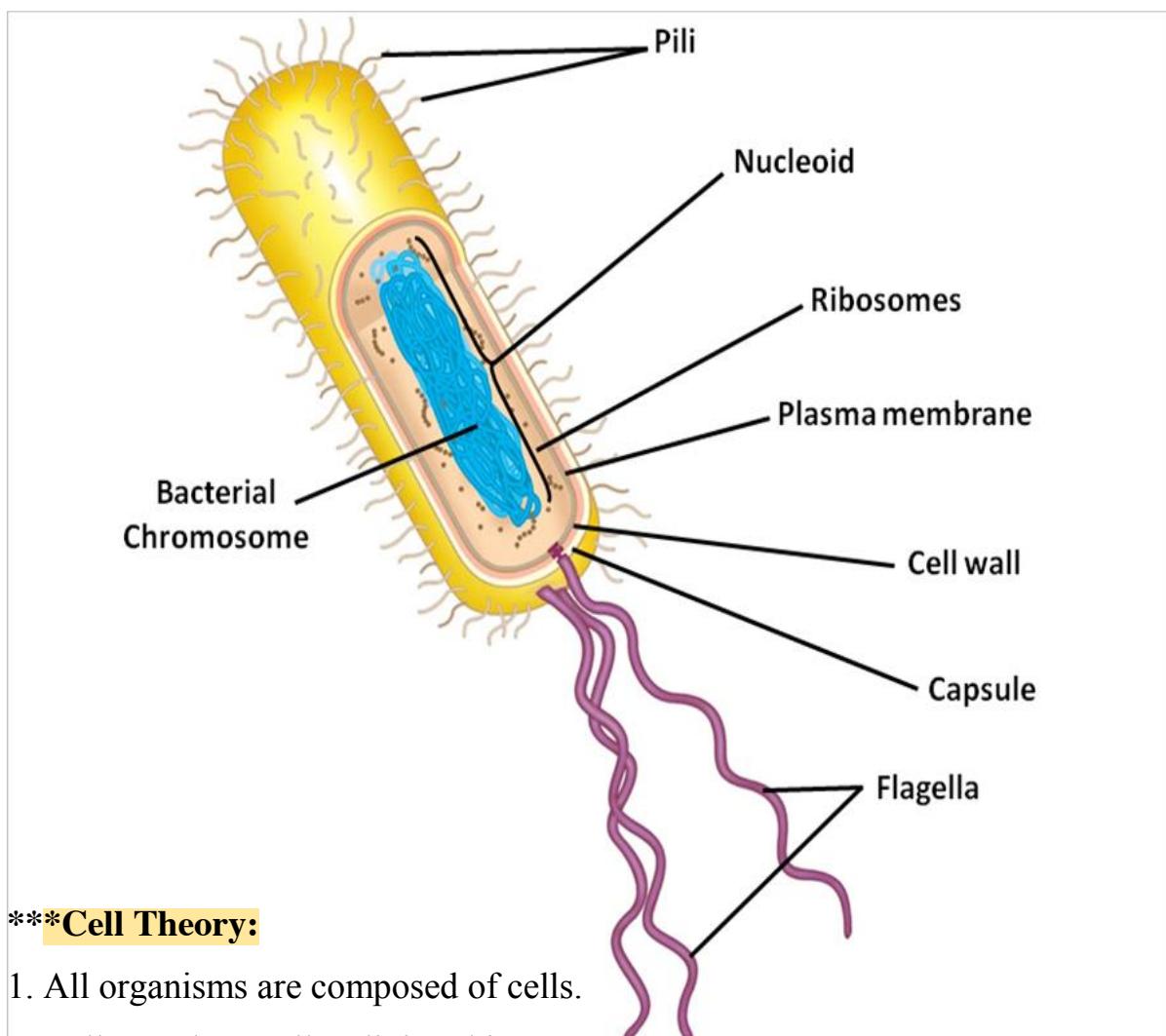
#### Spiral Form



### 4. Other Bacteria Shapes:

Trichome-forming, sheathed, stalked, filamentous, square, star-shaped, spindle-shaped, lobed and pleomorphic.

## IV- The Structure of a typical Bacterial Cell



### \*\*\*Cell Theory:

1. All organisms are composed of cells.
2. Cells are the smallest living things.
3. Cells arise only from pre-existing cells.

### \*\*\*All cells have certain structures in common.

1. Genetic material – in a nucleoid or nucleus.
2. Cytoplasm – a semifluid matrix.
3. Plasma membrane – a phospholipid bilayer.

## I- The genetic material (Nucleoid):

The genetic material of *bacteria* is present in the nucleoid. This nucleoid is not surrounded by a nuclear membrane and the genetic material is very simple in their structure and small in size.

## II- Capsule & Slime layer:

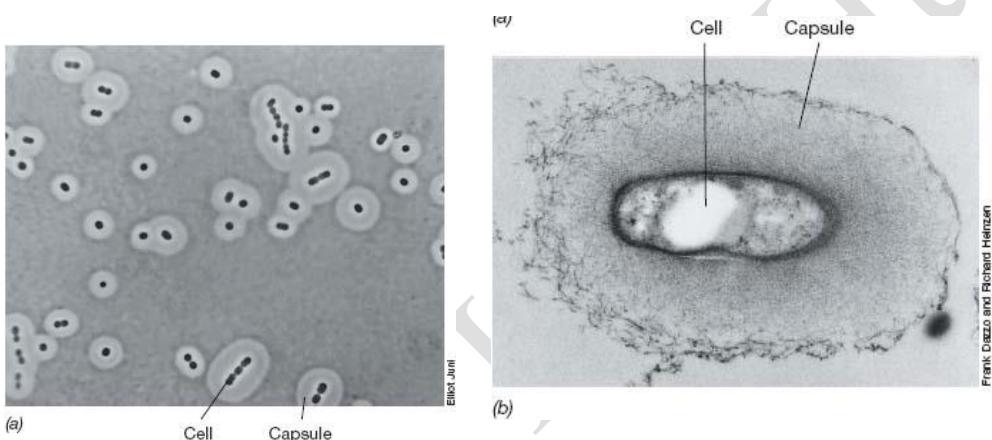
Many prokaryotic organisms secrete slimy or sticky materials on their cell surface. These materials consist of either polysaccharide or protein. These are not considered part of the cell wall because they do not confer significant structural strength on the cell. The terms “capsule” and “slime layer” are used to describe these layers.

Capsules and slime layers may be thick or thin and rigid or flexible, depending on their chemistry and degree of hydration. If the material is organized in a tight matrix that excludes small particles, such as India ink, it is called a **capsule** (Figure 2). If the material is more easily deformed, it will not exclude particles and is more difficult to see; this form is called a slime layer. In addition, capsules are typically firmly attached to the cell wall, whereas slime layers are loosely attached and can be lost from the cell surface.

Polysaccharide layers have several functions in bacteria. Surface polysaccharides assist in the attachment of microorganisms to solid surfaces. Pathogenic microorganisms that enter the animal body by specific routes usually do so by first binding specifically to surface components of host tissues. This binding is often mediated by surface polysaccharides on the bacterial cell. Many nonpathogenic *bacteria* also bind to solid surfaces in nature, sometimes forming a thick layer of cells

called a ***biofilm***. Extracellular polysaccharides play a key role in the development of biofilms.

Polysaccharide outer layers play other roles as well. For example, encapsulated pathogenic *bacteria* are typically more difficult for phagocytic cells of the immune system to recognize and subsequently destroy. In addition, because outer polysaccharide layers bind a significant amount of water, it is likely that these layers play some role in resistance of the cell to desiccation.



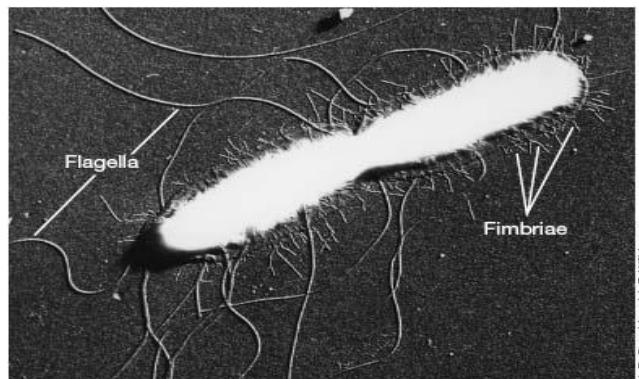
**Figure 2 Bacterial capsules.** (a) Capsules of *Acinetobacter* species observed by negative staining cells with India ink and phase-contrast microscopy. India ink does not penetrate the capsule and so the capsule appears as a light area surrounding the cell, which appears black. (b) Electron micrograph of a thin section of a cell of *Rhizobium trifolii* stained with ruthenium red to reveal the capsule. The diameter of the cell proper (not including the capsule) is about  $0.7\text{ }\mu\text{m}$ .

### III- Fimbriae & Pili:

Fimbriae and pili are filamentous structures composed of protein that extend from the surface of a cell and can have many functions. Fimbriae (Figure 3) enable organisms to stick to surfaces, including animal tissues in the case of some pathogenic bacteria, or to form pellicles (thin sheets of cells on a liquid surface) or biofilms on surfaces. Notorious among human pathogens in which these structures assist in the

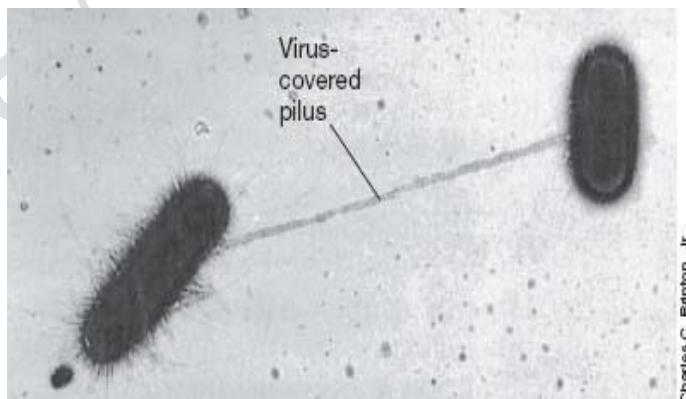
disease process include *Salmonella* species (salmonellosis), *Neisseria gonorrhoeae* (gonorrhea), and *Bordetella pertussis* (whooping cough).

**Figure 3 Fimbriae.** Electron micrograph of a dividing cell of *Salmonella typhi*, showing flagella and fimbriae. A single cell is about 0.9  $\mu\text{m}$  wide.



**Pili** are similar to fimbriae but are typically longer structures, and only one or a few pili are present on the surface of a cell. Because pili can be receptors for certain types of *viruses*, they can best be seen under the electron microscope when they become coated with virus particles (Figure 4). Although they may attach to surfaces as do fimbriae, pili also have other functions. A very important function is facilitating genetic exchange between prokaryotic cells in the process of conjugation.

**Figure 4 Pili.** The pilus on an *Escherichia coli* cell that is undergoing genetic transfer with a second cell is revealed by the viruses that have



Many classes of pili are known, distinguished by their structure and function. One class, called type IV pili, performs an unusual form of cell motility called **twitching motility**. Type IV pili are 6 nm in diameter and can extend for several micrometers away from the cell surface. Twitching motility is a type of gliding motility, movement along a solid surface. In

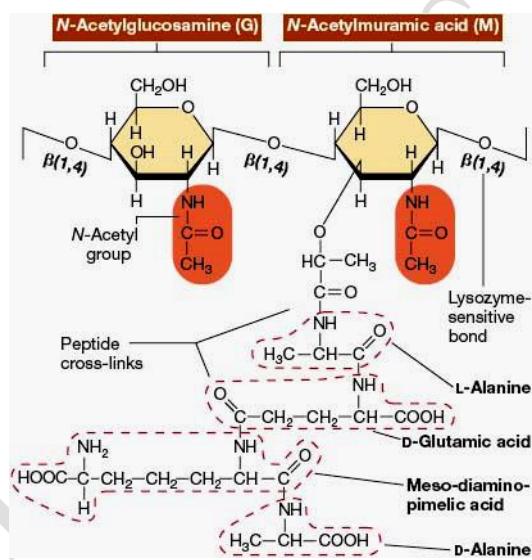
twitching motility, extension of the pili followed by their retraction drag the cell along a solid surface. Energy for twitching motility is supplied by ATP hydrolysis. Certain species of *Pseudomonas* and *Moraxella* are well known for their twitching motility.

Unlike other pili, type IV pili are present only at the poles of rod-shaped cells. Type IV pili have been implicated as key colonization factors for certain human pathogens, including *Vibrio cholerae* (*cholera*) and *Neisseria gonorrhoeae*. The ***twitching motility*** of these pathogens presumably assists the organisms in their movement across host tissues. Type IV pili are also thought to mediate genetic transfer by the process of ***transformation***.

## IV- Cell walls of Bacteria

### 4-1. The Cell Wall of *Bacteria*: Peptidoglycan

The cell walls of *Bacteria* have a rigid layer that is primarily responsible for the strength of the wall. In gram-negative *bacteria*, additional layers are present outside this rigid layer. The rigid layer, called **peptidoglycan**, is a polysaccharide composed of two sugar derivatives—*N*-acetylglucosamine (G) and *N*-acetylmuramic acid (M)—and a few amino acids, including L-alanine, D-alanine, D-glutamic acid, and either lysine or diaminopimelic acid (DAP) (Figure 4.1). These constituents are connected to form a repeating structure, the glycan tetrapeptide.

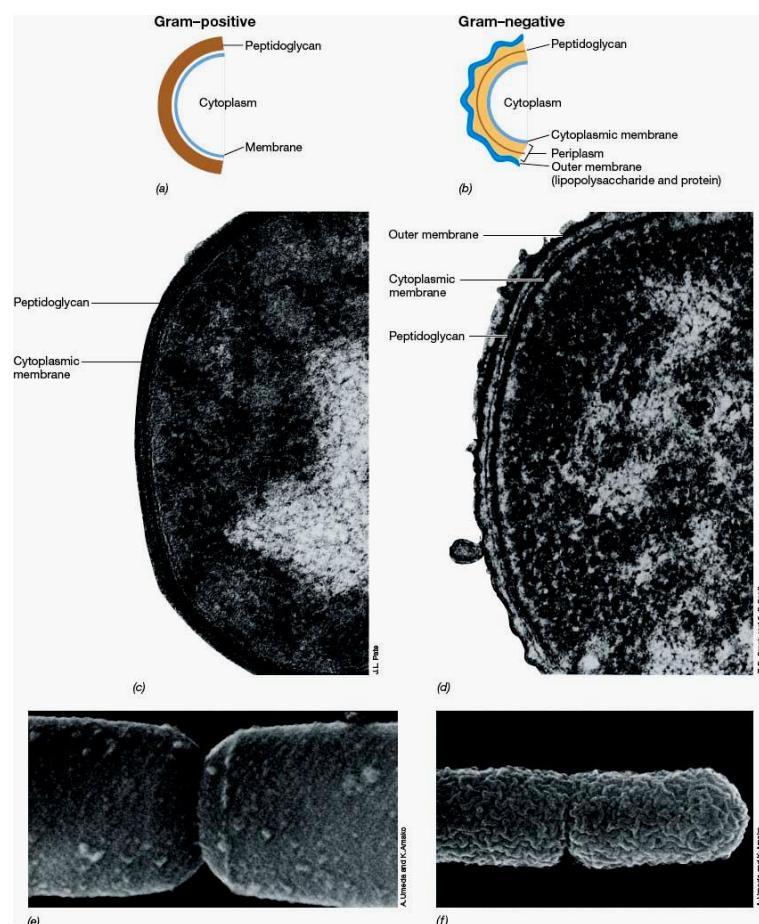


**Figure 4.1 Structure of the repeating unit in peptidoglycan, the glycan tetrapeptide.** The structure given is that found in *Escherichia coli* and most other gram-negative *Bacteria*. In some *Bacteria*, other amino acids are found.

Because of the activities of transport systems, the cytoplasm of bacterial cells maintains a high concentration of dissolved solutes. This causes significant osmotic pressure to develop—about 2 atmospheres in a bacterium such as *Escherichia coli*. This is roughly the same as the pressure in an automobile tire.

To withstand these pressures and prevent bursting—a process called *lysis*—*bacteria* have cell walls. Besides preventing osmotic *lysis*, cell walls also give shape and rigidity to the cell.

Species of *bacteria* can be divided into two major groups, called **gram-positive** and **gram-negative**. The distinction between gram-positive and gram-negative bacteria is based on the **Gram stain** reaction. But differences in cell wall structure are at the heart of the Gram-staining reaction. The appearance of the cell walls of gram-positive and gram-negative cells in the electron microscope differs markedly, as is shown in **Figure 4.2**. The gram-negative cell wall is a multilayered structure and quite complex, whereas the gram-positive cell wall is typically much thicker and consists almost entirely of a single type of molecule.

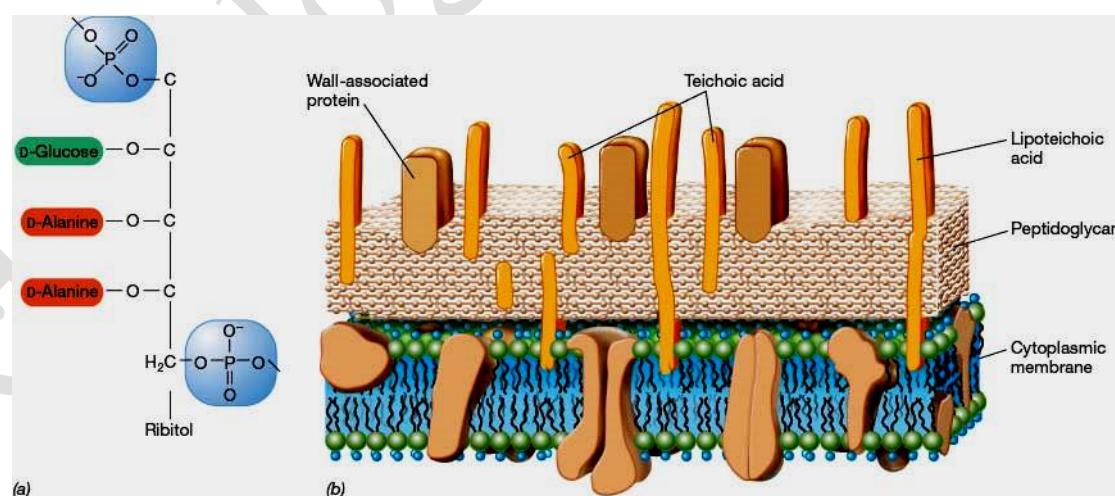


**Figure 4.2 Cell walls of Bacteria.** (a, b) Schematic diagrams of gram-positive and gram-negative cell walls. Transmission electron micrographs showing the cell wall of (c) a gram-positive bacterium, *Arthrobacter crystallopictes*, and (d) a gram-negative bacterium, *Leucothrix mucor*. (e, f) Scanning electron micrographs of gram-positive (*Bacillus subtilis*) and gram-negative (*Escherichia coli*) bacteria. Note differences in the surface texture in the cells shown in (e) and (f). A single cell of *B. subtilis* or *E. coli* is about 1  $\mu\text{m}$  wide.

## 4-2. The Gram-Positive Cell Wall

In gram-positive *bacteria*, as much as 90% of the cell wall consists of peptidoglycan. And, although some *bacteria* have only a single layer of peptidoglycan surrounding the cell, many *bacteria*, especially gram-positive *bacteria*, have several (up to about 25) sheets of peptidoglycan stacked one upon another.

Many gram-positive *bacteria* have acidic substances called **teichoic acids** embedded in their cell wall. Teichoic acids include all cell wall, cytoplasmic membrane, and capsular polymers containing glycerophosphate or ribitol phosphate residues. These polyalcohols are connected by phosphate esters and usually have other sugars and D-alanine attached (Figure 4.5a). Teichoic acids are covalently bonded to muramic acid residues in the cell wall peptidoglycan. Because they are negatively charged, teichoic acids are partially responsible for the negative charge of the cell surface. Teichoic acids also function to bind  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  for eventual transport into the cell. Certain teichoic acids are covalently bound to membrane lipids; thus they have been called lipoteichoic acids.

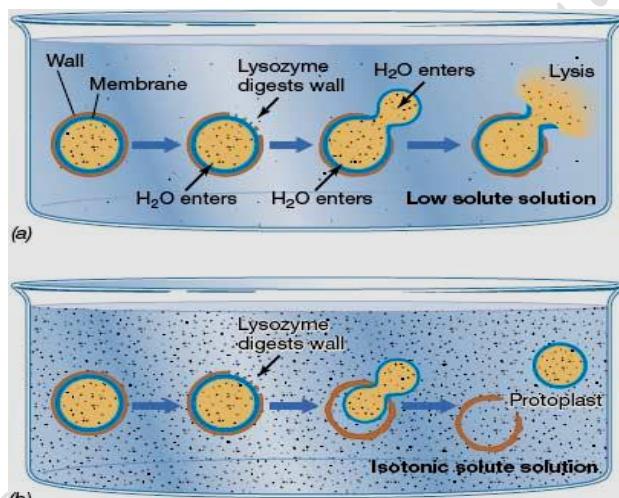


**Figure 4.5** Teichoic acids and the overall structure of the gram-positive bacterial cell wall. (a) Structure of the ribitol teichoic acid of *Bacillus subtilis*. The teichoic acid is a polymer of the repeating ribitol units shown here. (b) Summary diagram of the gram-positive bacterial cell wall.

Figure 4.5b summarizes the structure of the cell wall of gram-positive *Bacteria* and shows how teichoic acids and lipoteichoic acids are arranged in the overall wall structure.

### 4-3 Lysozyme and Protoplasts

Peptidoglycan can be destroyed by certain agents. One such agent is the enzyme *lysozyme*, a protein that breaks the  $\beta$ -1,4-glycosidic bonds between *N-acetylglucosamine* and *N-acetylmuramic acid* in peptidoglycan (Figure 4.1), thereby weakening the wall. Water then enters the cell and the cell swells and eventually bursts (*cell*



**Figure 4.6** Protoplasts and their formation. *Lysozyme* breaks the  $\beta$ -1,4 glycosidic bonds in peptidoglycan (Figure 4.3). (a) In dilute solutions, breakdown of the cell wall is immediately followed by cell lysis because the cytoplasmic membrane is structurally very weak. (b) In a solution containing an isotonic concentration of a solute such as sucrose, water does not enter the protoplast and it remains stable.

*lysis*) (Figure 4.6a). *Lysozyme* is found in animal secretions including tears, saliva, and other body fluids, and functions as a major line of defense against bacterial infection.

If a solute that does not penetrate the cell, such as sucrose, is added to a cell suspension containing *lysozyme*, the solute concentration outside the cell balances the concentration inside (these conditions are called *isotonic*). Under *isotonic* conditions, if *lysozyme* is used to digest *peptidoglycan*, water does not enter the cell and *lysis* does not occur. Instead, a **protoplast** (a bacterium that has lost its cell wall) is formed

(Figure 4.6b). If such sucrose-stabilized protoplasts are placed in water, they immediately *lyse*. The word spheroplast is often used as a synonym for protoplast, although the two words have slightly different meanings. **Protoplasts** are cells that are free of residual cell wall material, whereas **spheroplasts** contain pieces of wall material attached to the otherwise membrane-enclosed structure.

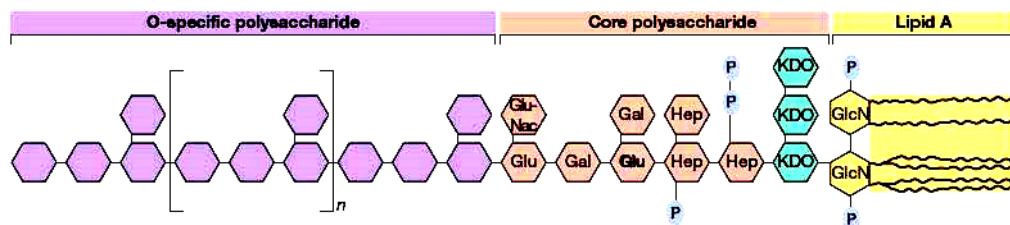
## 4-4. The Outer Membrane of Gram-Negative *Bacteria*

In gram-negative *bacteria* such as *Escherichia coli* only about 10% of the total cell wall consists of peptidoglycan. Instead, most of the cell wall is composed of the **outer membrane**. This layer is effectively a second lipid bilayer, but it is not constructed solely of phospholipid and protein as is the cytoplasmic membrane. The gram-negative cell outer membrane also contains polysaccharide. The lipid and polysaccharide are linked in the outer membrane to form a complex. Because of this, the outer membrane is called the **lipopolysaccharide** layer, or simply **LPS**.

### 4-4-1. Chemistry of LPS

The chemistry of LPS from several *bacteria* is known. As seen in Figure 4.7, the polysaccharide portion of LPS consists of two components, the core polysaccharide and the O-polysaccharide. In *Salmonella* species, where LPS has been best studied, the core polysaccharide consists of ketodeoxyoctonate (KDO), seven-carbon sugars (heptoses), glucose, galactose, and *N*-acetylglucosamine. Connected to the core is the O-polysaccharide, which typically contains galactose, glucose, rhamnose, and mannose (all hexoses), as well as one or more unusual dideoxy sugars such as abequose, colitose, paratose, or

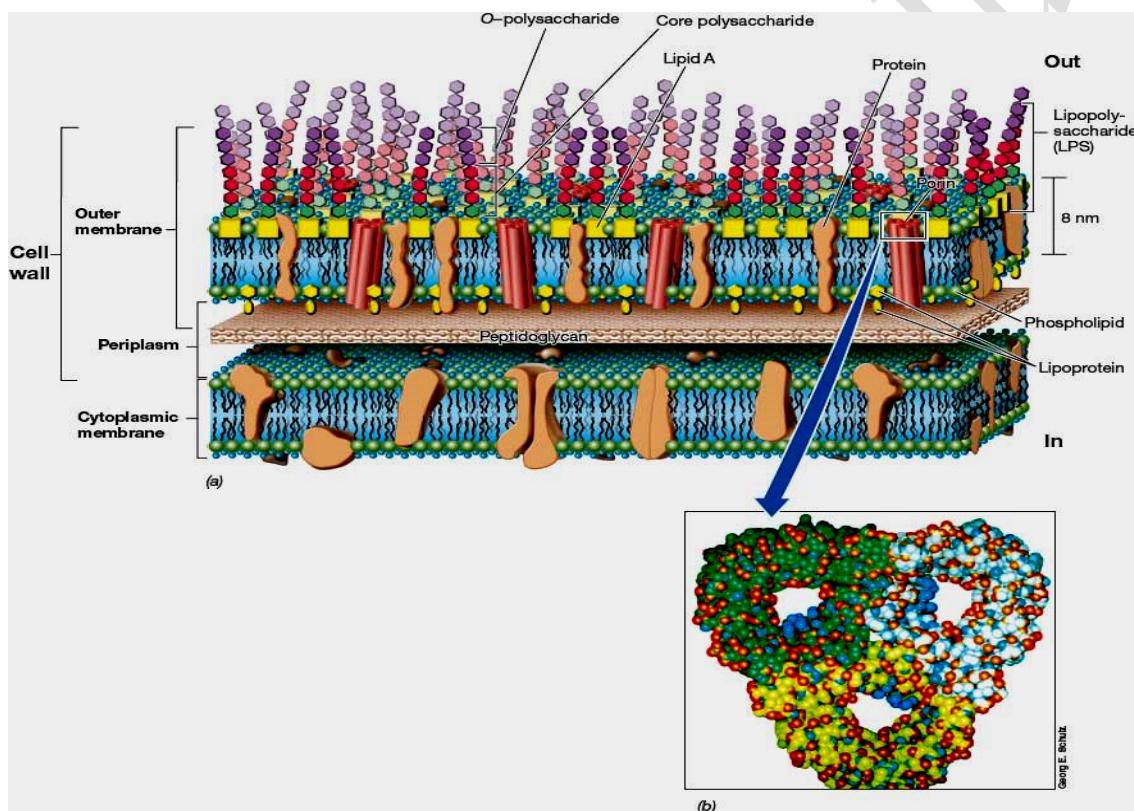
tyvelose. These sugars are connected in four- or five-membered sequences, which often are branched. When the sequences repeat, the long O-polysaccharide is formed.



**Figure 4.7** Structure of the lipopolysaccharide of gram-negative *Bacteria*. The chemistry of lipid A and the polysaccharide components varies among species of gram-negative *Bacteria*, but the major components (lipid A–KDO–core–O-specific) are typically the same. The O-specific polysaccharide varies greatly among species. KDO, ketodeoxyoctonate; Hep, heptose; Glu, glucose; Gal, galactose; GluNAc, N-acetylglucosamine; GlcN, glucosamine; P, phosphate. Glucosamine and the lipid A fatty acids are linked through the amine groups. The lipid A portion of LPS can be toxic to animals and comprises the endotoxin complex. Compare this figure with Figure 4.8 and follow the LPS components by the color-coding.

The relationship of the O-polysaccharide to the rest of the LPS is shown in Figure 4.8. The lipid portion of the LPS, called lipid A, is not a typical glycerol lipid (see Figure 4.7a), but instead the fatty acids are connected through the amine groups from a disaccharide composed of glucosamine phosphate (Figure 4.7). The disaccharide is attached to the core polysaccharide through KDO (Figure 4.7). Fatty acids commonly found in lipid A include caproic ( $C_6$ ), lauric ( $C_{12}$ ), myristic ( $C_{14}$ ), palmitic ( $C_{16}$ ), and stearic ( $C_{18}$ ) acids.

LPS replaces most of the phospholipids in the outer half of the outer membrane; the structure of the inner half more closely resembles that of the cytoplasmic membrane. However, a lipoprotein complex is also present on the inner half of the outer membrane (Figure 4.8a). Lipoprotein functions as an anchor between the outer membrane and peptidoglycan. Thus, although the outer membrane is considered a lipid bilayer, its structure is distinct from that of the cytoplasmic membrane, especially in the outer half in contact with the environment.



**Figure 4.8** The gram-negative cell wall. Note that although the outer membrane is often called the “second lipid bilayer,” the chemistry and architecture of this layer differ in many ways from that of the cytoplasmic membrane. (a) Arrangement of lipopolysaccharide, lipid A, phospholipid, porins, and lipoprotein in the outer membrane. See Figure 4.7 for details of the structure of LPS. (b) Molecular model of porin proteins. Note the four pores present, one within each of the proteins forming a porin molecule and a smaller central pore between the porin proteins. The view is perpendicular to the plane of the membrane. Model based on X-ray diffraction studies of *Rhodobacter blasticus* porin.

## **4-5. Relationship of Cell Wall Structure to the Gram Stain**

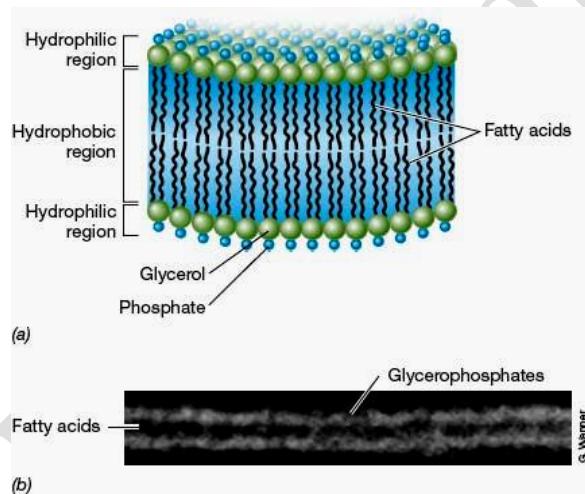
The structural differences between the cell walls of gram-positive and gram-negative *Bacteria* are thought to be responsible for differences in the Gram stain reaction. In the Gram stain, an insoluble crystal violet–iodine complex forms inside the cell. This complex is extracted by alcohol from gram-negative but not from gram-positive bacteria. As we have seen, gram-positive bacteria have very thick cell walls consisting of several layers of peptidoglycan (Figure 4.5); these become dehydrated by the alcohol, causing the pores in the walls to close and preventing the insoluble crystal violet–iodine complex from escaping. By contrast, in gram-negative bacteria, alcohol readily penetrates the lipid-rich outer membrane and extracts the crystal violet–iodine complex from the cell. After alcohol treatment, gram-negative cells are nearly invisible unless they are counterstained with a second dye, a standard procedure in the Gram stain.

## **V- The Cytoplasmic Membrane and Transport**

We now consider an extremely important cell structure, the cytoplasmic membrane, and review the major functions that the membrane has, in particular, in the transport of substances into and out of the cell.

## 5-1. The Cytoplasmic Membrane in *Bacteria*

The **cytoplasmic membrane** is a thin structure that surrounds the cell. Although very thin, this vital structure is the barrier separating the inside of the cell (the cytoplasm) from its environment. If the membrane is broken, the integrity of the cell is destroyed, the cytoplasm leaks into the environment, and the cell dies. The cytoplasmic membrane is also a highly selective permeability barrier, enabling a cell to concentrate specific metabolites and excrete waste materials.

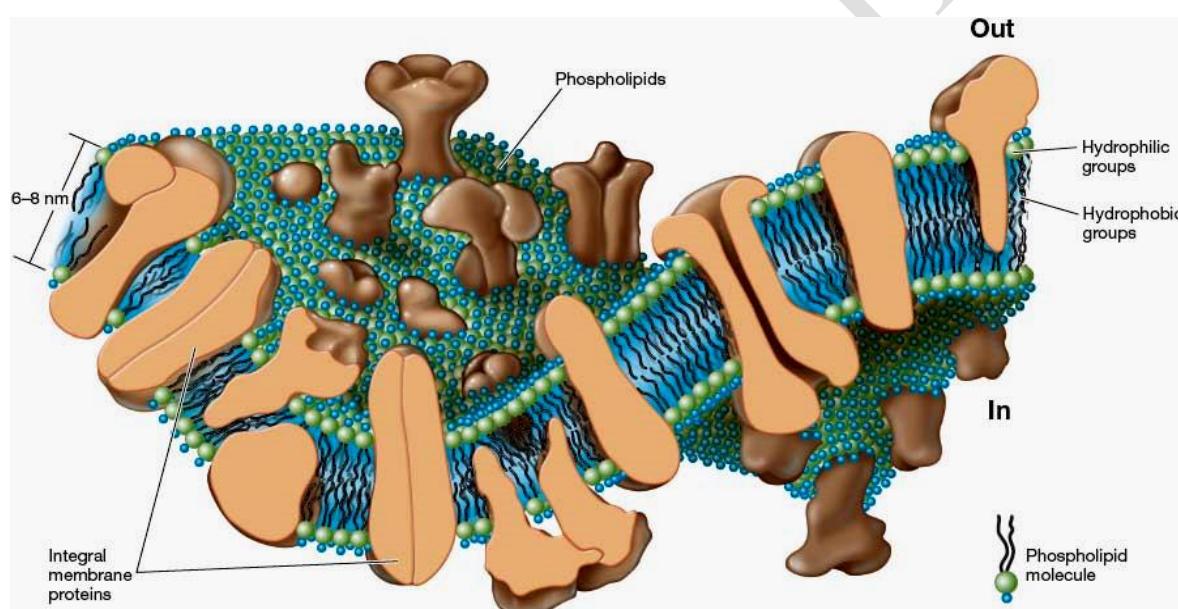


**Figure 5.1 Structure of a phospholipid bilayer.** (a) The general chemical structure of a phospholipid (b) Transmission electron micrograph of a membrane from the bacterium *Halorhodospira halochloris*. The dark inner area is the hydrophobic region of the model membrane shown in (a).

## 5-2. Composition of the Cytoplasmic Membranes

The general structure of biological membranes is a phospholipid bilayer (Figure 5.1). As previously discussed, phospholipids contain both hydrophobic (fatty acid) and hydrophilic (glycerol–phosphate) components and can exist in many different chemical forms as a result of variation in the groups attached to the glycerol backbone. As phospholipids aggregate in an aqueous solution, they naturally form bilayer structures. In a phospholipid membrane, the fatty acids point inward toward each other to form a hydrophobic environment, and the hydrophilic portions remain exposed to the external environment or the cytoplasm (Figure 5.1a).

The cytoplasmic membrane, which is 6–8 nanometers wide, can be seen with the electron microscope, where it appears as two light-colored lines separated by a darker area (Figure 5.1b). This unit membrane, as it is called (because each phospholipid leaf forms half of the “unit”), consists of a phospholipid bilayer with proteins embedded in it (Figure 5.2). The overall structure of the cytoplasmic membrane is stabilized by hydrogen bonds and hydrophobic interactions. In addition,  $Mg^{2+}$  and  $Ca^{2+}$  help stabilize the membrane by forming ionic bonds with negative charges on the phospholipids.



**Figure 5.2 Structure of the cytoplasmic membrane.** The inner surface (In) faces the cytoplasm and the outer surface (Out) faces the environment. Phospholipids compose the matrix of the cytoplasmic membrane, with the hydrophobic groups directed inward and the hydrophilic groups toward the outside, where they associate with water. Embedded in the matrix are proteins that are hydrophobic in the region that traverses the fatty acid bilayer. Hydrophilic proteins and other charged substances, such as metal ions, may attach to the hydrophilic surfaces. Although there are some chemical differences, the overall structure of the cytoplasmic membrane shown is similar in both *prokaryotes* and *eukaryotes*.

Although in a diagram the cytoplasmic membrane may appear rather rigid (Figure 5.2), in reality it is somewhat fluid, having a viscosity

approximating that of light-grade oil. Membrane biologists used to think that membranes were highly fluid, with proteins free to float around within a “sea” of lipid. We now know that this model is incorrect; some movement in the membrane is likely, although how extensive this is and how important it is to membrane function is unknown.

### 5-3. Membrane Proteins

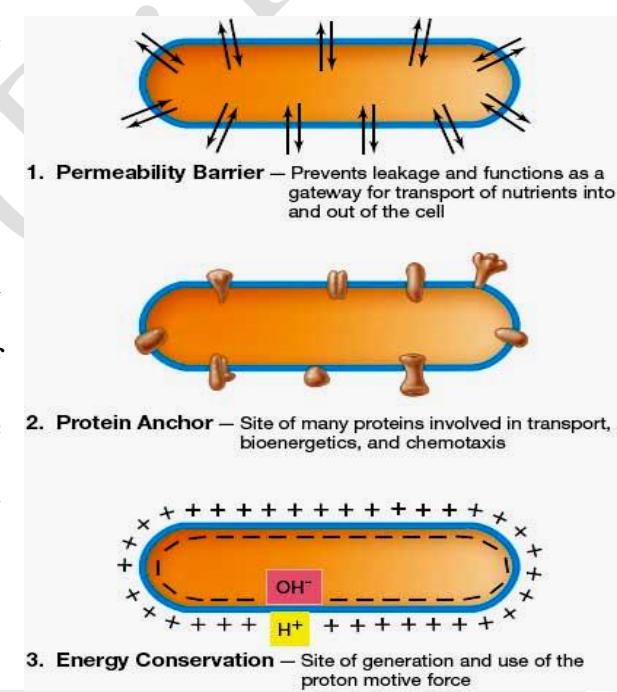
The major proteins of the cytoplasmic membrane have hydrophobic surfaces in their regions that span the membrane and hydrophilic surfaces in their regions that contact the environment and the cytoplasm (Figure 5.2). The outer surface of the cytoplasmic membrane faces the environment and in certain bacteria interacts with a variety of proteins that bind substrates or process large molecules for transport into the cell (periplasmic proteins). The inner side of the cytoplasmic membrane faces the cytoplasm and interacts with proteins involved in energy-yielding reactions and other important cellular functions.

Many membrane proteins are firmly embedded in the membrane and are called *integral membrane proteins*. Other proteins have one portion anchored in the membrane and extramembrane regions that point into or out of the cell (Figure 5.2). Still other proteins, called *peripheral membrane proteins*, are not embedded in the membrane at all but are nevertheless firmly associated with membrane surfaces. Some of these peripheral membrane proteins are lipoproteins, proteins that contain a lipid tail that anchors the protein into the membrane. These proteins typically interact with integral membrane proteins in important cellular processes such as energy metabolism and transport.

Proteins in the cytoplasmic membrane are arranged in patches (Figure 5.2); instead of being distributed evenly, proteins are clustered, a strategy that allows the grouping of proteins that interact or that have similar function. The overall protein content of the membrane is also quite high membrane proteins are indeed rather crowded—and it is thought that the lipid bilayer varies in thickness from 6 to 8 nm to accommodate thicker and thinner patches of proteins.

## 5-4.The Functions of Cytoplasmic Membranes

The cytoplasmic membrane is more than just a barrier separating the inside from the outside of the cell. The membrane plays critical roles in cell function. First and foremost, the membrane functions as a permeability barrier, preventing the passive leakage of substances into or out of the cell (Figure 5.3). Secondly, the membrane is an anchor for many proteins. Some of these are enzymes that catalyze bioenergetic reactions and others transport substances into and out of the cell. We will learn that the cytoplasmic membrane is also a major site of energy conservation in the cell. The membrane has an energetically charged form in which protons ( $H^+$ ) are separated from hydroxyl ions ( $OH^-$ ) across its surface. This charge separation is a form of energy, analogous to the potential energy present in a charged battery. This energy source, called the ***proton motive force***,

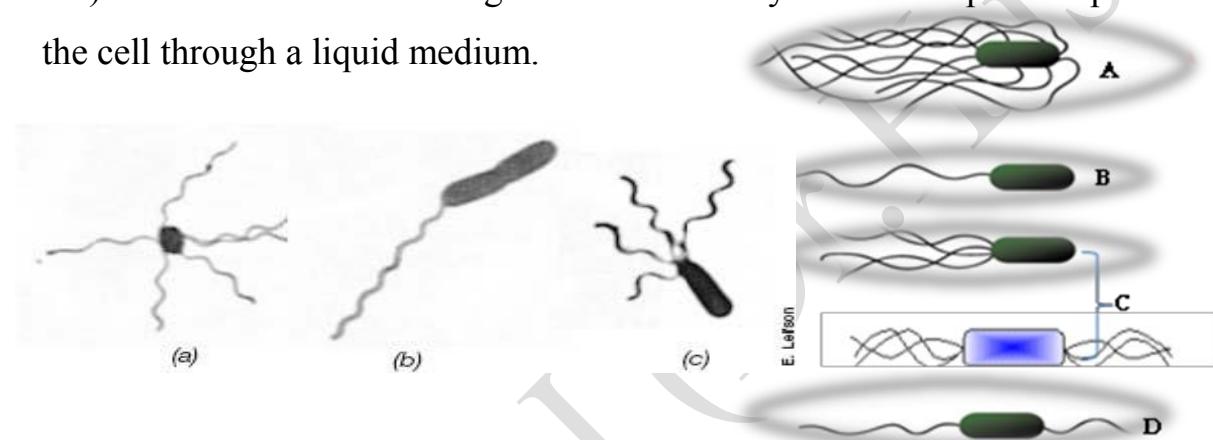


**Figure 5.3 The major functions of the cytoplasmic membrane.** Although structurally weak, the cytoplasmic membrane has many important cellular functions.

is responsible for driving many energy-requiring functions in the cell, including some forms of transport, motility, and biosynthesis of the cell's energy currency, ATP.

## VI- Flagella and Motility

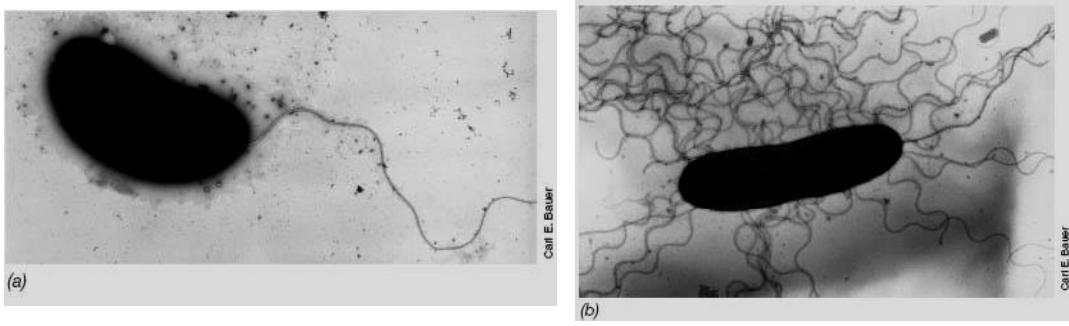
Many prokaryotes are motile by swimming, and this function is typically due to a structure called the **flagellum** (plural, flagella) (Figure 6.1). We see here that the flagellum functions by rotation to push or pull the cell through a liquid medium.



**Figure 6.1 Bacterial flagella.** Light photomicrographs of prokaryotes containing different arrangements of flagella. Cells are stained with Leifson flagella stain. (a) *Peritrichous*. (b) *Polar*. (c) *Lophotrichous*, (d) *Amphitrichous*.

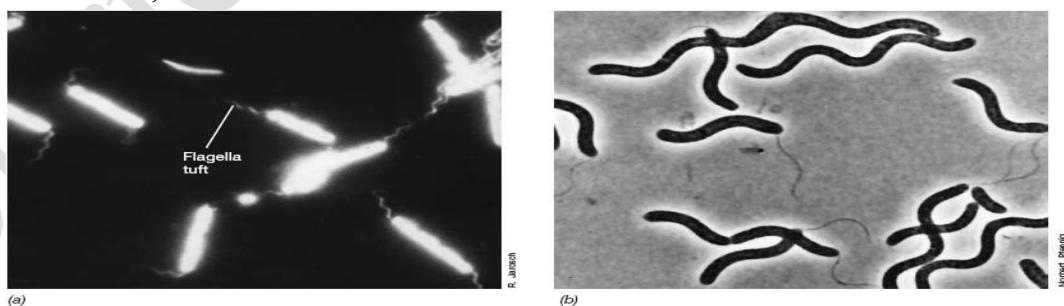
### 6.1- Flagella of Bacteria

Bacterial flagella are long, thin appendages free at one end and attached to the cell at the other end. Bacterial flagella are so thin (15–20 nm, depending on the species) that a single flagellum can be seen with the light microscope only after being stained with special stains that increase their diameter (Figure 6.2). However, flagella are easily seen with the electron microscope (Figure 6.2).



**Figure 6.2 Bacterial flagella as observed by negative staining in the transmission electron microscope.** (a) A single polar flagellum. (b) Peritrichous flagella. Both micrographs are of cells of the phototrophic bacterium *Rhodospirillum centenum*, which are about  $1.5\text{ }\mu\text{m}$  wide. Cells of *R. centenum* are normally polarly flagellated but under certain growth conditions form peritrichously flagellated “swarmer” cells.

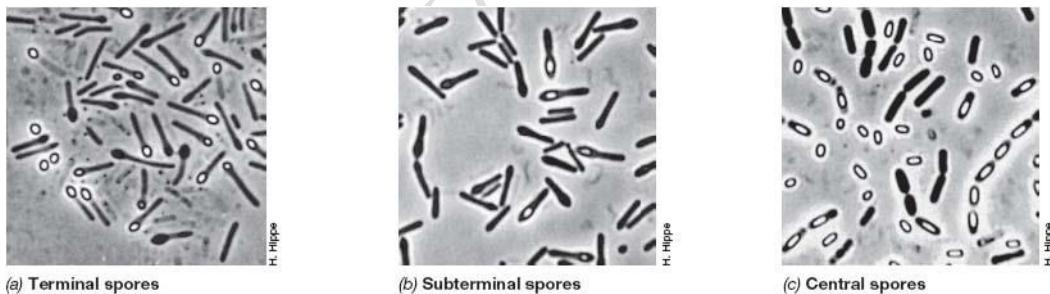
Flagella can be attached to cells in different patterns. In **polar flagellation**, the flagella are attached at one or both ends of the cell (Figures 6.1b and 6.2a). Occasionally a tuft (group) of flagella may arise at one end of the cell, a type of polar flagellation called **lophotrichous** (“lopho” means “tuft”; “trichous” means “hair”) (Figure 6.1c). Tufts of flagella of this type can be seen in living cells by dark-field microscopy (Figure 6.3a), where the flagella appear light and are attached to light-colored cells against a dark background. In relatively large prokaryotes, tufts of flagella can also be observed by phase-contrast microscopy (Figure 6.3b). When a tuft of flagella emerges from both poles, flagellation is called **amphitrichous**. In **peritrichous flagellation** (Figures 6.1a and 6.2b), flagella are inserted at many locations around the cell surface (“peri” means “around”). The type of flagellation, polar or peritrichous, is a characteristic used in the classification of bacteria.



**Figure 6.3 Bacterial flagella observed in living cells.** (a) Dark-field photomicrograph of a group of large rod-shaped bacteria with flagellar tufts at each pole. A single cell is about  $2\text{ }\mu\text{m}$  wide. (b) Phase-contrast photomicrograph of cells of the large phototrophic purple bacterium *Rhodospirillum photometricum* with lophotrichous flagella that emanate from one of the poles. A single cell measures about  $3 \times 30\text{ }\mu\text{m}$ .

## VII- Endospores

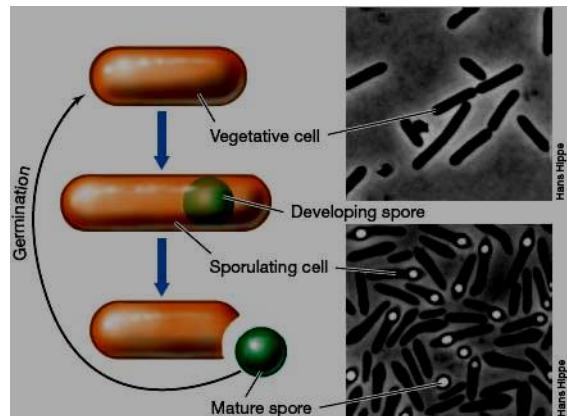
Certain species of *Bacteria* produce structures called **endospores** (Figure 7.1) during a process called sporulation. Endospores (the prefix “endo” means “within”) are highly differentiated cells that are extremely resistant to heat, harsh chemicals, and radiation. Endospores function as survival structures and enable the organism to endure difficult times, including but not limited to extremes of temperature, drying, or nutrient depletion. Endospores can thus be thought of as the dormant stage of a bacterial life cycle: vegetative cell → endospore → vegetative cell. Endospores are also ideal structures for dispersal of an organism by wind, water, or through the animal gut. Endospore-forming bacteria are found most commonly in the soil, and the genera *Bacillus* and *Clostridium* are the best studied of endospore-forming *bacteria*.



**Figure 7.1** The bacterial endospore. Phase-contrast photomicrographs illustrating endospore morphologies and intracellular locations in different species of endospore-forming *bacteria*.

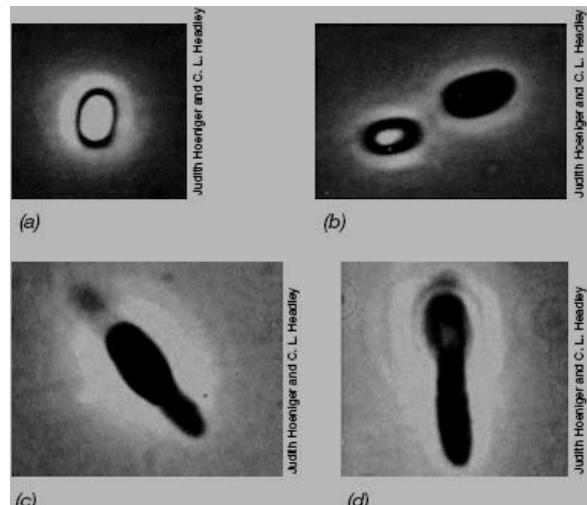
## 7-1. Endospore Formation and Germination

During endospore formation, a vegetative cell is converted into a nongrowing, heat-resistant structure (Figure 7.2). Cells do not sporulate when they are actively growing but only when growth ceases owing to the exhaustion of an essential nutrient. Thus, cells of *Bacillus*, a typical endospore-forming bacterium, cease vegetative growth and begin sporulation when, for example, a key nutrient such as carbon or nitrogen becomes limiting.



**Figure 7.2** The life cycle of an endospore-forming bacterium. The phase-contrast photomicrographs are of cells of *Clostridium pascui*. A cell is about 0.8  $\mu\text{m}$  wide.

An endospore can remain dormant for years, but it can convert back to a vegetative cell relatively rapidly. This process involves three steps: **activation, germination, and outgrowth** (Figure 7.3). Activation is most easily accomplished by heating freshly formed endospores for several minutes at an elevated but sublethal temperature. Activated endospores are then conditioned to germinate when placed in the presence of specific nutrients, such as certain amino acids (alanine is a

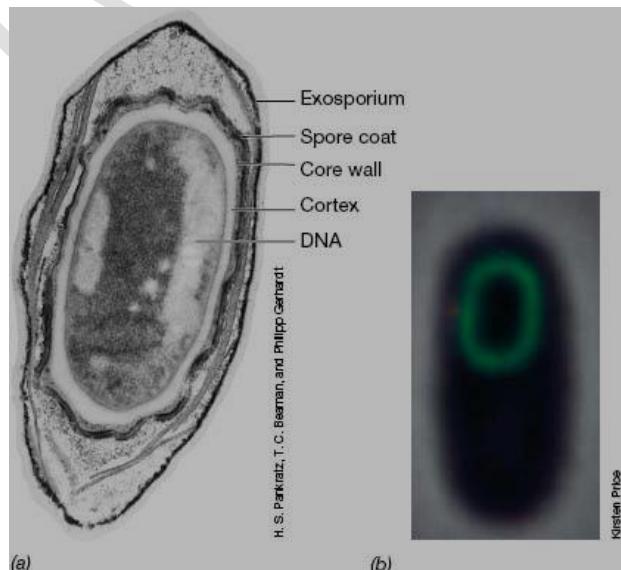


**Figure 7.3** Endospore germination in *Bacillus*. Conversion of an endospore into a vegetative cell. The series of photomicrographs shows the sequence of events starting from a highly refractile mature endospore (a). (b) (Activation) refractivity is being lost. (c), (d) The new vegetative cell is emerging (outgrowth).

particularly good trigger of endospore germination). Germination, usually a rapid process (on the order of several minutes), involves loss of microscopic refractivity of the endospore, increased ability to be stained by dyes, and loss of resistance to heat and chemicals. The final stage, outgrowth, involves visible swelling due to water uptake and synthesis of new RNA, proteins, and DNA. The cell emerges from the broken endospore and begins to grow (**Figure 4.40**). The cell then remains in vegetative growth until environmental signals once again trigger sporulation.

## 7-2. Endospore Structure

Endospores stand out under the light microscope as strongly refractile structures (see Figures 7.1 – 7.4). Endospores are impermeable to most dyes, so occasionally they are seen as unstained regions within cells that have been stained with basic dyes such as methylene blue. To stain endospores, special stains and procedures must be used. In the classical endospore-staining protocol, malachite green is used as a stain and is infused into the spore with steam.



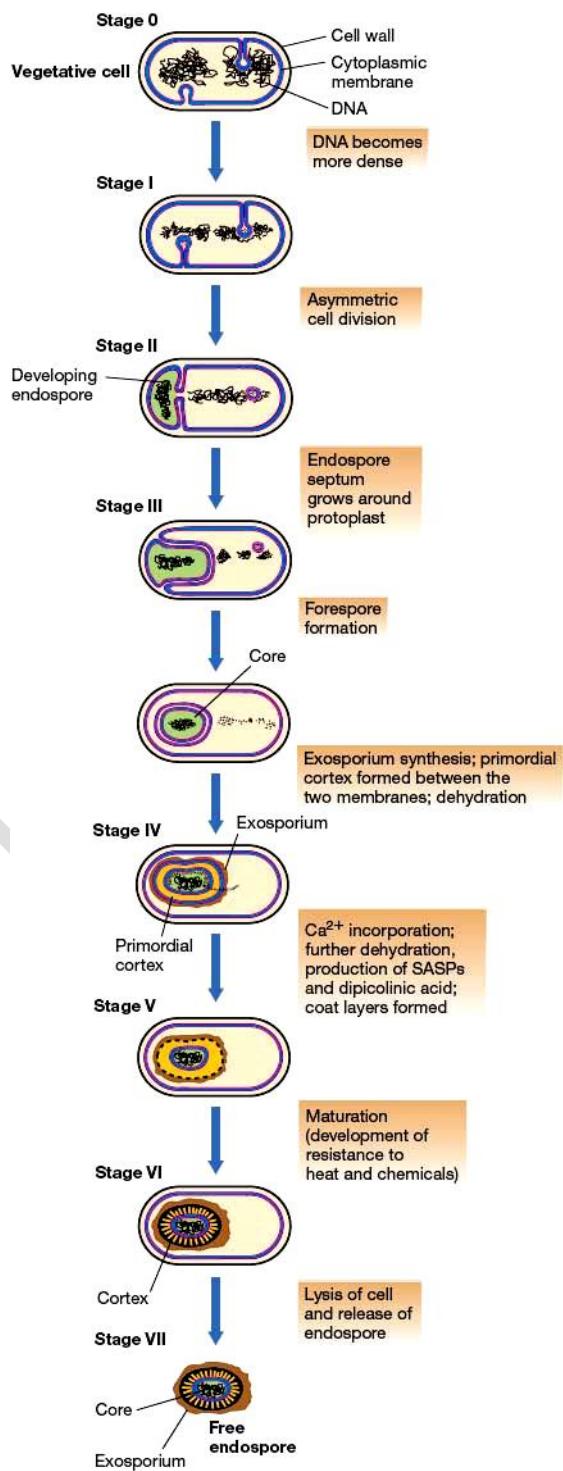
**Figure 7.4** Structure of the bacterial endospore. (a) Transmission electron micrograph of a thin section through an endospore of *Bacillus megaterium*. (b) Fluorescent photomicrograph of a cell of *Bacillus subtilis* undergoing sporulation. The green area is due to a dye that specifically stains a sporulation protein in the spore coat.

The structure of the endospore as seen with the electron microscope differs distinctly from that of the vegetative cell (Figure 7.4). In particular, the endospore is structurally more complex in that it has many layers that are absent from the vegetative cell. The outermost layer is the exosporium, a thin protein covering. Within this are the spore coats, composed of layers of spore-specific proteins (Figure 7.4b). Below the spore coat is the cortex, which consists of loosely cross-linked peptidoglycan, and inside the cortex is the core, which contains the core wall, cytoplasmic membrane, cytoplasm, nucleoid, ribosomes, and other cellular essentials. Thus, the endospore differs structurally from the vegetative cell primarily in the kinds of structures found outside the core wall.

One substance that is characteristic of endospores but absent from vegetative cells is **dipicolinic acid** (Figure 7.5a). This substance has been found in the endospores of all endospore-forming bacteria examined and is located in the core. Endospores are also enriched in calcium ( $\text{Ca}^{2+}$ ), most of which is complexed with dipicolinic acid (Figure 7.5b). The calcium–dipicolinic acid complex of the core represents about 10% of the dry weight of the endospore. The complex functions to reduce water availability within the endospore, thus helping to dehydrate it. In addition, the complex intercalates (inserts between bases) in DNA, and in so doing stabilizes DNA to heat denaturation.

## 7-4. The Sporulation Process

Sporulation is a complex series of events in cellular differentiation; many genetically directed changes in the cell underlie the conversion from vegetative growth to sporulation. The structural changes occurring in sporulating cells of *Bacillus* are shown in Figure 7.6. Sporulation can be divided into several stages. In *Bacillus subtilis*, where detailed studies have been done, the entire sporulation process takes about 8 hours and begins with asymmetric cell division (Figure 7.6). Genetic studies of mutants of *Bacillus*, each blocked at one of the stages of sporulation shown in Figure 7.6, indicate that more than 200 genes are specific to the process. Sporulation requires that the synthesis of many proteins needed for vegetative cell functions cease and that specific endospore proteins be made. This is accomplished by the activation of



**Figure 7.6** Stages in endospore formation. Stages 0 through VII are defined from genetic studies and microscopic analyses of sporulation in *Bacillus subtilis*.

several families of endospore-specific genes in response to an environmental trigger to sporulate. The proteins encoded by these genes catalyze the series of events leading from a moist, metabolizing, vegetative cell to a relatively dry, metabolically inert, but extremely resistant endospore (Table 4.3 and Figure 7.6).

**Table 4.3 Differences between endospores and vegetative cells**

Characteristic	Vegetative cell	Endospore
Structure	Typical gram-positive cell; a few gram-negative cells	Thick spore cortex; Spore coat; exosporium
Microscopic appearance	Nonrefractile	Refractile
Calcium content	Low	High
Dipicolinic acid	Absent	Present
Enzymatic activity	High	Low
Metabolism ( $O_2$ uptake)	High	Low or absent
Macromolecular synthesis	Present	Absent
mRNA	Present	Low or absent
DNA and ribosomes	Present	Present
Heat resistance	Low	High
Radiations resistance	Low	High
Resistance to chemicals (for example, $H_2O_2$ ) and acids	Low	High
Stainability by dyes	Stainable	Stainable only with special methods
Action of lysozyme	Sensitive	Resistant
Water content	High, 80–90%	Low, 10–25% in core
Small acid-soluble proteins (product of <i>ssp</i> genes)	Absent	Present
Cytoplasmic pH	About pH 7	About pH 5.5–6.0 (in core)

# **Chapter 2**

## **Bacterial Nutrition**

## Nutrition and Culture of Microorganisms

Before a cell can replicate, it must coordinate many different chemical reactions and organize molecules into specific structures. Collectively, these reactions are called **metabolism**. Metabolic reactions are either energy *releasing*, called **catabolic reactions (catabolism)**, or energy *requiring*, called **anabolic reactions (anabolism)**. Several catabolic and anabolic reactions occur in cells, and we will examine some of the key ones in this and future chapters. However, before we do, we consider how microorganisms are grown in the laboratory and the nutrients they need for growth. Indeed, most of what we know about the metabolism of microorganisms has emerged from the study of laboratory cultures. Our focus here will be on chemoorganotrophic microorganisms, also called *heterotrophs*; these are organisms that use organic compounds for carbon and energy. Later in this chapter we will briefly consider energy-generating mechanisms other than chemoorganotrophy, including chemolithotrophy and phototrophy.

### I- Microbial Nutrition

Besides water, cells consist mainly of macromolecules and that macromolecules are polymers of smaller units called monomers. Microbial nutrition is that aspect of microbial physiology that deals with the supply of monomers (or the precursors of monomers) that cells need for growth. Collectively, these required substances are called *nutrients*.

Different organisms need different complements of nutrients, and not all nutrients are required in the same amounts. Some nutrients, called

*macronutrients*, are required in large amounts, while others, called *micronutrients*, are required in trace amounts. All microbial nutrients originate from the chemical elements; however, just a handful of elements dominate biology (Figure 1). Although cells consist mainly of H, O, C, N, P, and S, at least 50 of the chemical elements are metabolized in some way by microorganisms (Figure 1). We begin our study of microbial nutrition with the key macronutrient elements, *carbon* and *nitrogen* (Figure 1 and Table 5.1).

Group →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Period ↓	1	H																He
	1	3	4															
2	Li		Be															
3		11	12															
4	Na		Mg	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
5	K	Ca		Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br
6	Rb	Sr	Y	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
	55	56		71	72	73	74	75	76	77	78	79	80	81	82	83	84	85
	Ca	Ba	Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn

Key:   
 Essential for all microorganisms  
 Essential cations and anions for most microorganisms  
 Trace metals, some essential for some microorganisms  
 Used for special functions  
 Unessential, but metabolized  
 Unessential, not metabolized

**Figure 1 A microbial periodic table of the elements.** With the exception of uranium, which can be metabolized by some prokaryotes, elements in period 7 or beyond in the complete periodic table of the elements are not metabolized. The atomic number of each element is shown in the upper right corner of each box.

## 1.1- Carbon and Nitrogen

All cells require carbon, and most prokaryotes require organic compounds as their source of carbon. On a dry weight basis, a typical cell is about 50% carbon, and carbon is the major element in all classes of macromolecules. *Bacteria* can assimilate organic compounds and use them to make new cell material. Amino acids, fatty acids, organic acids, sugars, nitrogen bases, aromatic compounds, and countless other organic compounds can be used by one or another bacterium. By contrast, some microorganisms are *autotrophs* and able to build all of their cellular

structures from carbon dioxide ( $\text{CO}_2$ ). The energy needed to support autotrophy is obtained from either light or inorganic chemicals.

Following carbon, the next most abundant element in the cell is nitrogen. A bacterial cell is about 12% nitrogen (by dry weight), and nitrogen is a key element in proteins, nucleic acids, and several other cell constituents. In nature, nitrogen is available in both organic and inorganic forms (Table 5.1). However, the bulk of available nitrogen is in inorganic form, either as ammonia ( $\text{NH}_3$ ), nitrate ( $\text{NO}_3^-$ ), or  $\text{N}_2$ . Virtually all *bacteria* can use ammonia as their nitrogen source, and many can also use nitrate. However, nitrogen gas ( $\text{N}_2$ ) can satisfy the nitrogen needs of only a few *bacteria*, the nitrogen-fixing *bacteria*.

## 1.2- Other Macronutrients: P, S, K, Mg, Ca, Na

After C and N, other essential elements are needed in smaller amounts. Phosphorus occurs in nature in the form of organic and inorganic phosphates and is required by the cell primarily for synthesis of nucleic acids and phospholipids. Sulfur is required because of its structural role in the amino acids cysteine and methionine and because it is present in several vitamins, including thiamine, biotin, and lipoic acid, as well as in coenzyme A. Most sulfur originates from inorganic sources in nature, either sulfate ( $\text{SO}_4^{2-}$ ) or sulfide ( $\text{HS}^-$ ) (Table 5.1).

Potassium is required by all organisms. Many enzymes, including some necessary for protein synthesis, require potassium for activity. Magnesium functions to stabilize ribosomes, membranes, and nucleic acids and is also required for the activity of many enzymes. Calcium helps stabilize cell walls in many microorganisms and plays a key role in the heat stability of endospores. Sodium is required by some but not all

microorganisms and is typically a reflection of the habitat. For example, seawater contains relatively high levels of  $\text{Na}^+$ , and marine microorganisms usually require sodium for growth. By contrast, freshwater species are usually able to grow in the absence of sodium.

**Table 5.1 Macronutrients**

Element	Usual form found in the environment
Carbon (C)	$\text{CO}_2$ , organic compounds
Hydrogen (H)	$\text{H}_2\text{O}$ , organic compounds
Oxygen (O)	$\text{H}_2\text{O}$ , $\text{O}_2$ , organic compounds
Nitrogen (N)	$\text{NH}_3$ , $\text{NO}_3^-$ , $\text{N}_2$ , organic nitrogen compounds
Phosphorus (P)	$\text{PO}_4^{3-}$
Sulfur (S)	$\text{H}_2\text{S}$ , $\text{SO}_4^{2-}$ , organic S compounds, metal sulfides ( $\text{FeS}$ , $\text{CuS}$ , $\text{ZnS}$ , $\text{NiS}$ , and so on)
Potassium (K)	$\text{K}^+$ in solution or as various K salts
Magnesium (Mg)	$\text{Mg}^{2+}$ in solution or as various Mg salts
Sodium (Na)	$\text{Na}^+$ in solution or as $\text{NaCl}$ or other Na salts
Calcium (Ca)	$\text{Ca}^{2+}$ in solution or as $\text{CaSO}_4$ or other Ca salts
Iron (Fe)	$\text{Fe}^{2+}$ or $\text{Fe}^{3+}$ in solution or as $\text{FeS}$ , $\text{Fe(OH)}_3$ , or many other Fe salts

### 1.3- Iron and Trace Metals

Microorganisms require various metals for growth. Chief among these is iron, which plays a major role in cellular respiration. Iron is a key component of cytochromes and iron–sulfur proteins involved in electron transport reactions. Under anoxic conditions, iron is generally in the ferrous ( $\text{Fe}^{2+}$ ) form and soluble. However, under oxic conditions, iron is typically in the ferric ( $\text{Fe}^{3+}$ ) form in insoluble minerals. To obtain iron from such minerals, cells produce iron-binding agents called **siderophores** that bind iron and transport it into the cell. One major

group of siderophores consists of derivatives of hydroxamic acid, which chelate ferric iron strongly. Once the iron–hydroxamate complex has passed into the cell, the iron is released and the hydroxamate can be excreted and used again for iron transport.

Many other metals are required or otherwise metabolized by microorganisms. Like iron, these micronutrients are called *trace elements*. Micronutrients typically play a role as components of enzymes, the cells' catalysts. Table 5.2 lists the major micronutrients and examples of enzymes in which each plays a role.

**Table 5.2** **Micronutrients (trace elements) needed by microorganisms<sup>a</sup>**

<b>Element</b>	<b>Cellular function</b>
Boron (B)	Present in an autoinducer for quorum sensing in bacteria; also found in some polyketide antibiotics
Chromium (Cr)	Required by mammals for glucose metabolism; microbial requirement possible but not proven
Cobalt (Co)	Vitamin B <sub>12</sub> ; transcarboxylase (propionic acid bacteria)
Copper (Cu)	Respiration, cytochrome c oxidase; photosynthesis, plastocyanin, some superoxide dismutases
Iron (Fe) <sup>b</sup>	Cytochromes; catalases; peroxidases; iron–sulfur proteins; oxygenases; all nitrogenases
Manganese (Mn)	Activator of many enzymes; present in certain superoxide dismutases and in the water-splitting enzyme in oxygenic phototrophs (photosystem II)
Molybdenum (Mo)	Certain flavin-containing enzymes; some nitrogenases, nitrate reductases, sulfite oxidases, DMSO-TMAO reductases; some formate dehydrogenases
Nickel (Ni)	Most hydrogenases; coenzyme F <sub>430</sub> of methanogens; carbon monoxide dehydrogenase; urease
Selenium (Se)	Formate dehydrogenase; some hydrogenases; the amino acid selenocysteine
Tungsten (W)	Some formate dehydrogenases; oxotransferases of hyperthermophiles
Vanadium (V)	Vanadium nitrogenase; bromoperoxidase
Zinc (Zn)	Carbonic anhydrase; alcohol dehydrogenase; RNA and DNA polymerases; and many DNA-binding proteins

<sup>a</sup>Not every micronutrient listed is required by all cells; some metals listed are found in enzymes present in only specific microorganisms.

<sup>b</sup>Needed in greater amounts than other trace metals.

## 1.4- Growth Factors

Growth factors are organic compounds that share with trace metals the fact that they are required in only small amounts and then only by certain organisms. Growth factors include vitamins, amino acids, purines, and pyrimidines. Although most microorganisms are able to synthesize all of these compounds, some require one or more of them preformed from the environment and thus must be supplied with these compounds when cultured in the laboratory.

Vitamins are the most commonly required growth factors. Most vitamins function as coenzymes, and these are summarized in Table 5.3. Vitamin requirements vary among microorganisms, ranging from none to several. Lactic acid *bacteria*, which include the genera *Streptococcus*, *Lactobacillus*, and *Leuconostoc*, among others, are renowned for their many vitamin requirements, which are even more extensive than those of humans (see Table 5.4).

**Table 5.3** Growth factors: Vitamins and their functions

Vitamin	Function
p-Aminobenzoic acid	Precursor of folic acid
Folic acid	One-carbon metabolism; methyl group transfer
Biotin	Fatty acid biosynthesis; $\beta$ -decarboxylations; some $\text{CO}_2$ fixation reactions
Cobalamin ( $\text{B}_{12}$ )	Reduction of and transfer of single carbon fragments; synthesis of deoxyribose
Lipoic acid	Transfer of acyl groups in decarboxylation of pyruvate and $\alpha$ -ketoglutarate
Nicotinic acid (niacin)	Precursor of $\text{NAD}^+$ (see Figure 5.11); electron transfer in oxidation-reduction reactions
Pantothenic acid	Precursor of coenzyme A; activation of acetyl and other acyl derivatives
Riboflavin	Precursor of FMN (see Figure 5.16), FAD in flavoproteins involved in electron transport
Thiamine ( $\text{B}_1$ )	$\alpha$ -Decarboxylations; transketolase
Vitamins $\text{B}_6$ (pyridoxal-pyridoxamine group)	Amino acid and keto acid transformations
Vitamin K group; quinones	Electron transport; synthesis of sphingolipids
Hydroxamates	Iron-binding compounds; solubilization of iron and transport into cell

## **II- 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 a microorganism, careful attention must be paid to both the selection and preparation of media for successful culture to take place.

### **2.1- Classes of Culture Media**

Two broad classes of culture media are used in microbiology: **defined media** and **complex media** (Table 5.4). Defined media are prepared by adding precise amounts of highly purified inorganic or organic chemicals to distilled water. Therefore, the *exact* chemical composition of a defined medium is known. Of major importance in any culture medium is the carbon source, since all cells need large amounts of carbon to make new cell material. In a simple defined medium (Table 5.4), a single carbon source is usually present. The nature of the carbon source and its concentration depends on the organism to be cultured. For growing many organisms, knowledge of the exact composition of a medium is not essential. In these instances complex media may suffice and may even be advantageous. Complex media employ digests of animal or plant products, such as casein (milk protein), beef (beef extract), soybeans (tryptic soy broth), yeast cells (yeast extract), or any of a number of other highly nutritious yet impure substances. These digests are commercially available in powdered form and can be quickly weighed and dissolved in distilled water to yield a medium. However, a major concession in using a complex medium is loss of control over its precise nutrient composition.

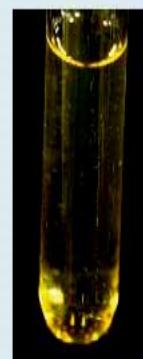
In particular situations, especially in clinical microbiology, culture media are often made to be selective or differential (or both). A *selective* medium contains compounds that selectively inhibit the growth of some microorganisms but not others. By contrast, a *differential* medium is one in which an indicator, typically a dye, is added that allows for the differentiation of particular chemical reactions that have occurred during growth. Differential media are quite useful for distinguishing between species of *bacteria*, some of which may carry out the particular reaction while others do not.

**Table 5.4 Examples of culture media for microorganisms with simple and demanding nutritional requirements<sup>a</sup>**

Defined culture medium for <i>Escherichia coli</i>	Defined culture medium for <i>Leuconostoc mesenteroides</i>	Complex culture medium for either <i>E. coli</i> or <i>L. mesenteroides</i>	Defined culture medium for <i>Thiobacillus thioparus</i>
K <sub>2</sub> HPO <sub>4</sub> 7 g	K <sub>2</sub> HPO <sub>4</sub> 0.6 g	Glucose 15 g	KH <sub>2</sub> PO <sub>4</sub> 0.5 g
KH <sub>2</sub> PO <sub>4</sub> 2 g	KH <sub>2</sub> PO <sub>4</sub> 0.6 g	Yeast extract 5 g	NH <sub>4</sub> Cl 0.5 g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> 1 g	NH <sub>4</sub> Cl 3 g	Peptone 5 g	MgSO <sub>4</sub> 0.1 g
MgSO <sub>4</sub> 0.1 g	MgSO <sub>4</sub> 0.1 g	KH <sub>2</sub> PO <sub>4</sub> 2 g	CaCl <sub>2</sub> 0.05 g
CaCl <sub>2</sub> 0.02 g	Glucose 25 g	Distilled water 1,000 ml	KCl 0.5 g
Glucose 4–10 g	Sodium acetate 25 g	pH 7	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> 2 g
Trace elements (Fe, Co, Mn, Zn, Cu, Ni, Mo) 2–10 µg each	Amino acids (alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine) 100–200 µg of each		Trace elements (same as <i>E. coli</i> recipe)
Distilled water 1,000 ml	Purines and pyrimidines (adenine, guanine, uracil, xanthine) 10 mg of each		Distilled water 1,000 ml pH 7
pH 7	Vitamins (biotin, folate, nicotinic acid, pyridoxal, pyridoxamine, pyridoxine, riboflavin, thiamine, pantothenate, p-aminobenzoic acid) 0.01–1 mg of each		Carbon source CO <sub>2</sub> from air
	Trace elements (see first column) 2–10 µg each		
	Distilled water 1,000 ml		
	pH 7		



(a)



(b)

<sup>a</sup>The photos are tubes of (a) the defined medium described, and (b) the complex medium described. Note how the complex medium is colored from the various organic extracts and digests that it contains. Photos courtesy of Cheryl L. Brodine and John Vercillo, Southern Illinois University at Carbondale.

# Major Nutritional Types of Microorganisms

Major nutritional type	Sources of energy, hydrogen/electrons, and carbon	Representative microorganisms
<b><i>Photoautotroph</i></b> <i>(Photolithotroph)</i>	Light energy, <b>inorganic</b> hydrogen/electron(H/e <sup>-</sup> ) donor, CO <sub>2</sub> carbon source	<i>Algae, Purple and green bacteria, Cyanobacteria</i>
<b><i>Photoheterotroph</i></b> <i>(Photoorganotroph)</i>	Light energy, <b>inorganic</b> H/e <sup>-</sup> donor, Organic carbon source	<i>Purple nonsulfur bacteria, Green sulfur bacteria</i>
<b><i>Chemoautotroph</i></b> <i>(Chemolithotroph)</i>	Chemical energy source ( <b>inorganic</b> ), Inorganic H/e <sup>-</sup> donor, CO <sub>2</sub> carbon source	<i>Sulfur-oxidizing bacteria, Hydrogen bacteria, Nitrifying bacteria</i>
<b><i>Chemoheterotroph</i></b> <i>(Chemoorganotroph)</i>	Chemical energy source ( <b>organic</b> ), Organic H/e <sup>-</sup> donor, Organic carbon source	<b>Most bacteria, fungi, protozoa</b>

# Chapter 3

## Bacterial Growth

# Microbial Growth

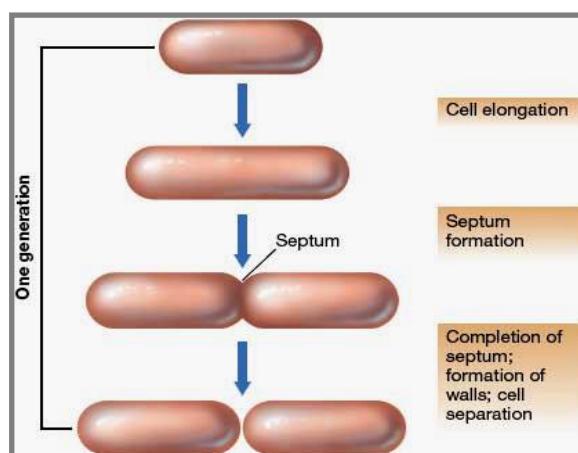
## 3.1- Cell Growth and Binary Fission

In microbiology, **growth** is defined as an increase in the number of cells. Microbial cells have a finite life span, and a species is maintained only as a result of continued growth of its population. Many practical situations call for the control of microbial growth. Knowledge of how microbial populations can rapidly expand is useful for designing methods to control microbial growth.

Bacterial cell growth depends upon a large number of chemical reactions of a wide variety of types. Some of these reactions transform energy. Others synthesize small molecules—the building blocks of macromolecules. Still others provide the various cofactors and coenzymes needed for enzymatic reactions. However, the main reactions of cell synthesis are the polymerization reactions that make macromolecules from monomers. As macromolecules accumulate in the cytoplasm of a cell, they are assembled into new structures, such as the cell wall, cytoplasmic membrane, flagella, ribosomes, inclusion bodies, enzyme complexes, and so on, eventually leading to cell division.

### 3.1-1 Binary Fission

In a growing rod-shaped cell, elongation continues until the cell divides into two new cells. This process is called **binary fission**



**Figure 1** Binary fission in a rod-shaped prokaryote. Cell numbers double every generation.

(“binary” to express the fact that two cells have arisen from one). In a growing culture of a rod-shaped *bacterium* such as *Escherichia coli*, cells elongate to approximately twice their original length and then form a partition that separates the cell into two daughter cells (Figure 1). This partition is called a septum and results from the inward growth of the cytoplasmic membrane and cell wall from opposing directions, which continues until the two daughter cells are pinched off. By definition, when one cell divides to form two, one generation has occurred, and the time required for this process is called the **generation time** (Figure 1).

In the period of one generation, all cellular constituents increase proportionally; cells are thus said to be in balanced growth. Each daughter cell receives a chromosome and sufficient copies of ribosomes and all other macromolecular complexes, monomers, and inorganic ions to exist as an independent cell. Partitioning of the replicated DNA molecule between the two daughter cells depends on the DNA remaining attached to the cytoplasmic membrane during division, with septum formation leading to separation of the chromosomes, one to each daughter cell.

The time required for a generation in a given bacterial species is highly variable and is dependent on both nutritional and genetic factors. Under the best nutritional conditions the generation time of a laboratory culture of *E. coli* is about 20 min. A few *bacteria* can grow even faster than this, but many grow much slower. In nature it is likely that microbial cells grow much slower than their maximum rate, because rarely are all conditions and resources necessary for optimal growth present at the same time.

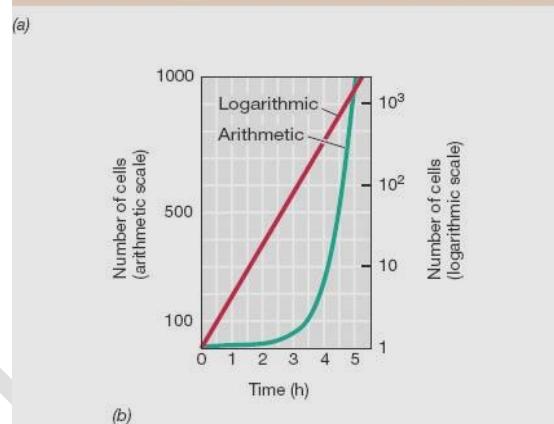
## 3.2- Growth Terminology and the Concept of Exponential Growth

During cell division one cell becomes two. During the time that it takes for this to occur (the generation time) both total cell number and mass double. Generation times vary widely among microorganisms. In general, most *bacteria* have shorter generation times than do most microbial eukaryotes. The generation time of a given organism in culture is dependent on the growth medium and the incubation conditions used. Many *bacteria* have minimum generation times of 0.5–6 h under the best of growth conditions, but a few very rapidly growing organisms are known whose doubling times are less than 20 min and a few slow-growing organisms whose doubling times are as long as several days or even weeks.

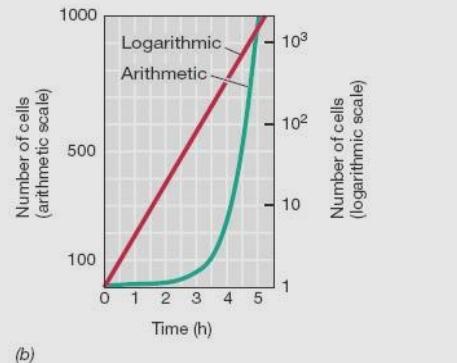
### 3.3- Exponential Growth

A growth experiment beginning with a single cell having a doubling time of 30 min is presented in Figure 2. This pattern of population increase, where the number of cells doubles during a constant time interval, is called **exponential growth**. When the cell number from such an experiment is graphed on arithmetic (linear) coordinates as a

Time (h)	Total number of cells	Time (h)	Total number of cells
0	1	4	256 ( $2^8$ )
0.5	2	4.5	512 ( $2^9$ )
1	4	5	1,024 ( $2^{10}$ )
1.5	8	5.5	2,048 ( $2^{11}$ )
2	16	6	4,096 ( $2^{12}$ )
2.5	32	.	.
3	64	.	.
3.5	128	10	1,048,576 ( $2^{19}$ )



**Figure 2 The rate of growth of a microbial culture.** (a) Data for a population that doubles every 30 min. (b) Data plotted on arithmetic (left ordinate) and logarithmic (right ordinate) scales.



function of time, one obtains a curve with a continuously increasing slope (Figure 2b).

By contrast, when the number of cells is plotted on a logarithmic ( $\log_{10}$ ) scale and time is plotted arithmetically (a semilogarithmic graph), as shown in Figure 2b, the points fall on a straight line. This straight-line function reflects the fact that the cells are growing exponentially—the cell population is doubling in a constant time interval. Semilogarithmic graphs are also convenient and simple to use to estimate generation times from a set of growth data.

### 3.4- The Consequences of Exponential Growth

During exponential growth, the increase in cell number is initially rather slow but increases at an ever faster rate. In the later stages of growth, this results in an explosive increase in cell numbers. For example, in the experiment in Figure 2, the rate of cell production in the first 30 min of growth is 1 cell per 30 min. However, between 4 and 4.5 h of growth, the rate of cell production is considerably higher, 256 cells per 30 min, and between 5.5 and 6 h of growth it is 2,048 cells per 30 min (Figure 2). Thus in an actively growing bacterial culture, cell numbers can get very large very quickly.

Consider the following practical implication of exponential growth. For a nonsterile and nutrient-rich food product such as milk to stand under ideal bacterial growth conditions for a few hours during the early stages of exponential growth, when total cell numbers are relatively low, is not detrimental. However, standing for the same length of time during the later stages of growth, when cell numbers are initially much higher, is disastrous. The lactic acid bacteria responsible for milk spoilage

contaminate milk during its collection. These harmless organisms are held in check by refrigeration temperatures ( $\sim 4^{\circ}\text{C}$ ), and only after several days of slow growth and lactic acid production are the effects of spoilage (rancid milk) noticeable. But at room temperature or above, rapid growth of the same organisms accelerates the spoilage process and overnight, the milk can be spoiled.

### 3.5- The Microbial Growth Cycle

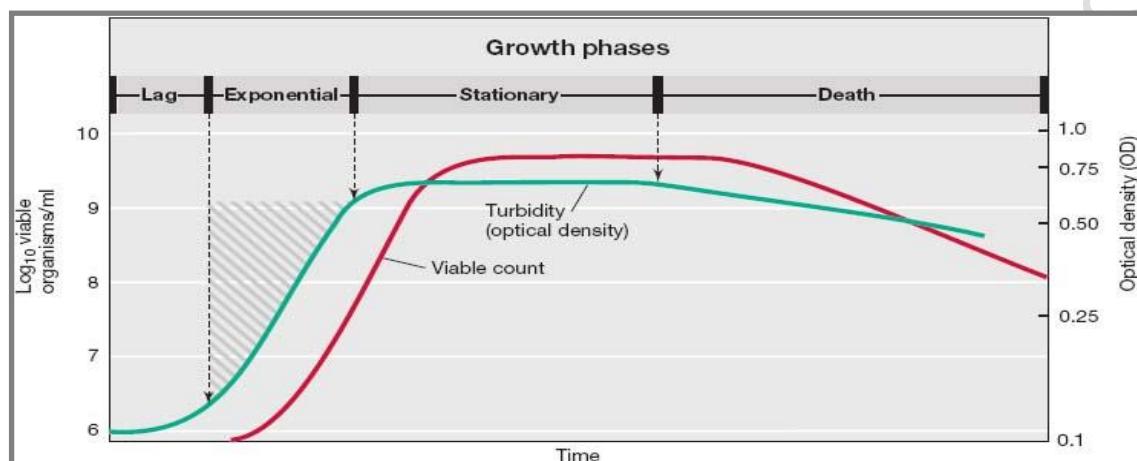
The data presented in Figure 2 reflect only part of the growth cycle of a microbial population, the part called *exponential growth*. For a culture growing in an enclosed vessel, such as a tube or a flask, a condition called a **batch culture**, exponential growth cannot continue indefinitely. Instead, a typical growth curve for a population of cells is obtained, as illustrated in [Figure 3](#). The growth curve describes an entire growth cycle, including the *lag phase*, *exponential phase*, *stationary phase*, and *death phase*.

#### **Lag Phase**

When a microbial population is inoculated into a fresh medium, growth usually begins only after a period of time called the *lag phase*. This interval may be brief or extended, depending on the history of the inoculum and the growth conditions. If an exponentially growing culture is transferred into the same medium under the same conditions of growth, there is no lag and exponential growth begins immediately. However, if the inoculum is taken from an old (*stationary phase*) culture and transferred into the same medium, there is usually a lag even if all the cells in the inoculum are alive. This is because the cells are depleted of various essential constituents and time is required for their biosynthesis.

A lag also ensues when the inoculum consists of cells that have been damaged (but not killed) by treatment with heat, radiation, or toxic chemicals because of the time required for the cells to repair the damage.

A lag is also observed when a microbial population is transferred from a rich culture medium to a poorer one; for example, from a complex



**Figure 3 Typical growth curves for a bacterial population.** A viable count measures the cells in the culture that are capable of reproducing. Optical density (turbidity), a quantitative measure of light scattering by a liquid culture, increases with the increase in cell number.

medium to a defined medium. To grow in any culture medium the cells must have a complete complement of enzymes for synthesis of the essential metabolites not present in that medium. Hence, upon transfer to a medium where essential metabolites must be biosynthesized, time is needed for production of the new enzymes that will carry out these reactions.

### ***Exponential Phase***

As we saw in the previous section, during the exponential phase of growth each cell divides to form two cells, each of which also divides to form two more cells, and so on, for a brief or extended period, depending on the available resources and other factors. Cells in exponential growth

are typically in their healthiest state and hence are most desirable for studies of their enzymes or other cell components.

Rates of exponential growth vary greatly. The rate of exponential growth is influenced by environmental conditions (temperature, composition of the culture medium), as well as by genetic characteristics of the organism itself. In general, prokaryotes grow faster than eukaryotic microorganisms, and small eukaryotes grow faster than large ones. This should remind us of the previously discussed concept of surface-to-volume ratio. Recall how small cells have an increased capacity for nutrient and waste exchange compared with larger cells, and this metabolic advantage can greatly affect their growth and other properties.

### ***Stationary Phase***

In a batch culture, such as in a tube or a flask, exponential growth is limited. Consider the fact that a single *bacterium* with a 20-min generation time would produce, if allowed to grow exponentially in a batch culture for 48 h, a population of cells that weighed 4000 times the weight of Earth! This is particularly impressive when it is considered that a single bacterial cell weighs only about one-trillionth ( $10^{-12}$ ) of a gram.

Obviously, this scenario is impossible. Something must happen to limit the growth of the population. Typically, either or both of two things limit growth: (1) an essential nutrient of the culture medium is used up, or (2) a waste product of the organism accumulates in the medium and inhibits growth. Either way, exponential growth ceases or the population reaches the stationary phase.

In the *stationary phase*, there is no net increase or decrease in cell number and thus the growth rate of the population is zero. Although the population may not grow during the stationary phase, many cell functions can continue, including energy metabolism and biosynthetic processes. In some cases there may be some cell division during the stationary phase but no net increase in cell number occurs. This is because some cells in the population grow, whereas others die, the two processes balancing each other out. This is a phenomenon called *cryptic growth*.

### ***Death Phase***

If incubation continues after a population reaches the *stationary phase*, the cells may remain alive and continue to metabolize, but they will eventually die. When this occurs, the population enters the *death phase* of the growth cycle. In some cases death is accompanied by actual cell lysis. Figure 8 indicates that the death phase of the growth cycle is also an exponential function. Typically, however, the rate of cell death is much slower than the rate of exponential growth.

The phases of bacterial growth shown in Figure 8 are reflections of the events in a population of cells, not in individual cells. Thus the terms lag phase, exponential phase, stationary phase, and death phase have no meaning with respect to individual cells but only to cell populations. Growth of an individual cell is a necessary prerequisite for population growth. But it is population growth that is most relevant to the ecology of microorganisms, because measurable microbial activities require microbial populations, not just an individual microbial cell.

## **3.6- Environmental Factors Affecting Microbial Growth**

The activities of microorganisms are greatly affected by the chemical and physical state of their environment. Many environmental factors can be considered. However, four key factors control the growth of all microorganisms: temperature, pH, water availability, and oxygen, and we consider these here. Some other factors can potentially affect the growth of microorganisms, such as pressure and radiation. These more specialized environmental factors will be considered later in this book when we encounter microbial habitats in which they play major roles.

### **3.6.1- Effect of Temperature on Microbial Growth**

Temperature is probably the most important environmental factor affecting the growth and survival of microorganisms. At either too cold or too hot a temperature, microorganisms will not be able to grow and may even die. The minimum and maximum temperatures for growth vary greatly among different microorganisms and usually reflect the temperature range and average temperature of their habitats.

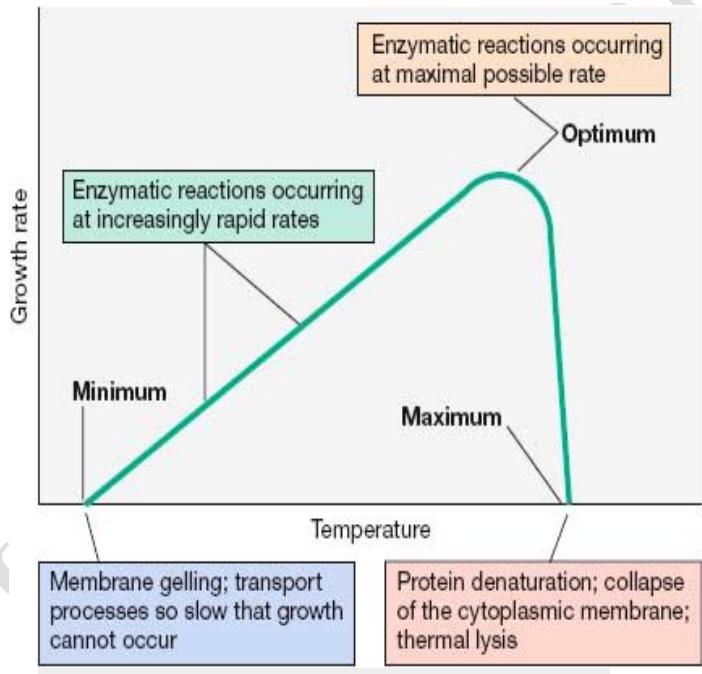
#### **3.6.1.1- Cardinal Temperatures**

Temperature affects microorganisms in two opposing ways. As temperatures rise, chemical and enzymatic reactions in the cell proceed at more rapid rates and growth becomes faster; however, above a certain temperature, cell components may be irreversibly damaged. Thus, as the temperature is increased within a given range, growth and metabolic function increase up to a point where denaturation reactions set in. Above this point, cell functions fall to zero.

For every microorganism there is thus a minimum temperature below which growth is not possible, an optimum temperature at which

growth is most rapid, and a maximum temperature above which growth is not possible (Figure 4). The optimum temperature is always nearer the maximum than the minimum. These three temperatures, called the **cardinal temperatures**, are characteristic for any given microorganism.

The cardinal temperatures of different microorganisms differ widely; some organisms have temperature optima as low as 4°C and some higher than 100°C. The temperature range throughout which microorganisms grow is even wider than this, from below freezing to well above the boiling point of water. However, no single organism can grow over this whole temperature range, as the range for any given organism is typically 25–40 degrees.



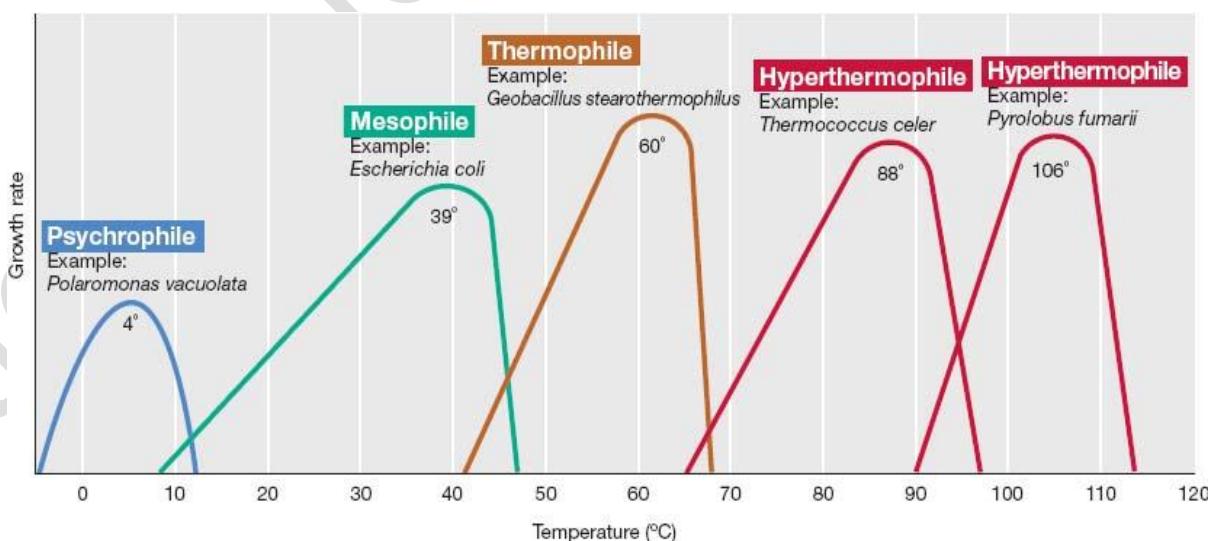
**Figure 4** The cardinal temperatures: Minimum, optimum, and maximum. The actual values may vary greatly for different organisms.

The maximum growth temperature of an organism reflects the temperature above which denaturation of one or more essential cell components, such as a key enzyme, occurs. The factors controlling an organism's minimum growth temperature are not as clear. However, as previously discussed, the cytoplasmic membrane must be in a fluid state for transport and other important functions to take place. An organism's minimum temperature may well be governed by membrane functioning: If an organism's cytoplasmic membrane stiffens to the point that it no longer functions properly in nutrient transport or can no longer develop a

proton motive force, the organism cannot grow. The growth temperature optimum reflects a state in which all or most cellular components are functioning at their maximum rate.

### 3.6.1.2- Temperature Classes of Organisms

Although there is a continuum of organisms, from those with very low temperature optima to those with high temperature optima, it is possible to distinguish at least four groups of microorganisms in relation to their growth temperature optima: **psychrophiles**, with low temperature optima; **mesophiles**, with midrange temperature optima; **thermophiles**, with high temperature optima; and **hyperthermophiles**, with very high temperature optima (Figure 5). Mesophiles are widespread in nature. They are found in warm-blooded animals and in terrestrial and aquatic environments in temperate and tropical latitudes. Psychrophiles and thermophiles are found in unusually cold and unusually hot environments, respectively. Hyperthermophiles are found in extremely hot habitats such as hot springs, geysers, and deep-sea hydrothermal vents.



**Figure 5** Temperature and growth relations in different temperature classes of microorganisms. The temperature optimum of each example organism is shown on the graph.

*Escherichia coli* is a typical mesophile, and its cardinal temperatures have been precisely defined. The optimum temperature for most strains of *E. coli* is 39°C, the maximum is 48°C, and the minimum is 8°C. Thus, the temperature range for *E. coli* is 40 degrees, near the high end for prokaryotes (Figure 5). We now turn to the interesting cases of microorganisms growing at very low or very high temperatures—the *extremophiles*.

### 3.6.4- Effect of pH on the Microbial Growth

Acidity or alkalinity of a solution is expressed by its **pH** on a scale on which neutrality is pH 7 (Figure 6). pH values less than 7 are acidic and those greater than 7 are alkaline. It is important to remember that pH is a logarithmic function—a change of 1 pH unit corresponds to a tenfold change in hydrogen ion concentration. Thus, vinegar (pH near 2) and household ammonia (pH near 11) differ in hydrogen ion concentration by a billionfold.

Every microorganism has a pH range within which growth is possible and typically shows a well-defined growth pH optimum. Most organisms show a growth pH range of 2–3 units. Most natural environments have pH values between 4 and 9, and organisms with optima in this range are most commonly encountered. Only a few species can grow at pH values of lower than 3 or greater than 9.

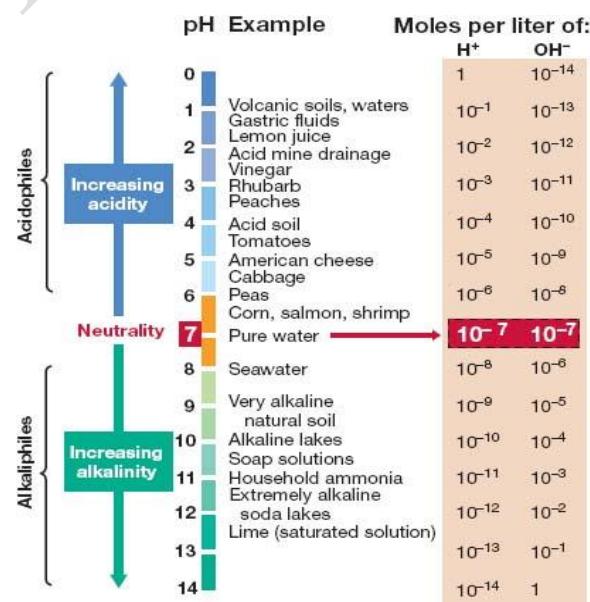


Figure 6 The pH scale. Although some microorganisms can live at very low or very high pH, the cell's internal pH remains near neutrality.

#### 3.6.4.1- Acidophiles

Organisms that grow optimally at low pH, typically below pH 6, are called ***acidophiles***. Fungi as a group tend to be more acid tolerant than *bacteria*. Many *fungi* grow best at pH 5 or below, and a few grow well at pH values as low as 2. Many *prokaryotes* are also *acidophilic*. In fact, some of these are obligate *acidophiles*, unable to grow at neutral pH. Obligately acidophilic *bacteria* include species of *Acidithiobacillus* and several genera of *Archaea*, including *Sulfolobus*, *Thermoplasma*, and *Ferroplasma*.

A critical factor governing acidophily is the stability of the cytoplasmic membrane. When the pH is raised to neutrality, the cytoplasmic membranes of strongly acidophilic *bacteria* are destroyed and the cells lyse. This indicates that these organisms are not just acid tolerant but that high concentrations of hydrogen ions are actually required for membrane stability. For example, the most acidophilic prokaryote known, *Picrophilus oshimae* (*Archaea*), grows optimally at pH 0.7. Above pH 4, cells of *P. oshimae* spontaneously lyse. *P. oshimae* inhabits extremely acidic thermal soils associated with volcanic activity.

### **3.6.4.2- Alkaliphiles**

A few *extremophiles* have very high pH optima for growth, sometimes as high as pH 11. Microorganisms showing growth pH optima of 9 or higher are called ***alkaliphiles***. *Alkaliphilic* microorganisms are typically found in highly alkaline habitats, such as soda lakes and high-carbonate soils. The most well-studied *alkaliphilic prokaryotes* have been *Bacillus* species, such as *Bacillus firmus*. This organism is *alkaliphilic* but has an unusually broad pH range for growth, from 7.5 to 11. Some extremely *alkaliphilic bacteria* are also *halophilic* (salt loving), and most

of these are *Archaea*. Many *phototrophic purple bacteria* are also strongly *alkaliphilic*. Some *alkaliphiles* have industrial uses because they produce hydrolytic enzymes, such as *proteases* and *lipases*, which function well at alkaline pH and are used as supplements for laundry detergents.

*Alkaliphiles* are of basic science interest for several reasons but particularly because of the bioenergetic problems they face living at such high pH. For example, how can a proton motive force be established when the external surface of the cytoplasmic membrane is so alkaline? In *B. firmus* it has been shown that a sodium ( $\text{Na}^+$ ) motive force rather than a proton motive force drives transport reactions and motility. Remarkably, however, a proton motive force is also established in cells of *B. firmus*, even though the external membrane surface is awash in hydroxyl ions. The proton motive force in *B. firmus* is responsible for driving respiratory ATP synthesis, just as it is in nonalkaliphiles.

### 3.6.4.3- Internal Cell pH

The optimal pH for growth of any organism is a measure of the pH of the extracellular environment only. The intracellular pH must remain relatively close to neutrality to prevent destruction of macromolecules in the cell. For the majority of microorganisms whose pH optimum for growth is between pH 6 and 8, organisms called *neutrophiles*, the cytoplasm remains neutral or very nearly so. However, in *acidophiles* and *alkaliphiles* the internal pH can vary from neutrality. For example, in the previously mentioned *acidophile* *P. oshimae*, the internal pH has been measured at pH 4.6, and in extreme *alkaliphiles* an intracellular pH of as high as 9.5 has been measured. If these are not the lower and upper limits

of cytoplasmic pH, respectively, they are extremely close to the limits. This is because DNA is acid-labile and RNA is alkaline-labile; if a cell cannot maintain these key macromolecules in a stable state, it obviously cannot survive.

### 3.6.5- Effect of Oxygen on the Microbial Growth

Because animals require molecular oxygen ( $O_2$ ), it is easy to assume that all organisms require oxygen. However, this is not true; many microorganisms can, and some must live in the total absence of oxygen.

Oxygen is poorly soluble in water, and because of the constant respiratory activities of microorganisms in aquatic or other moist habitats,  $O_2$  can quickly become exhausted. Thus, anoxic microbial habitats are common in nature and include muds and other sediments, bogs and marshes, water-logged soils, intestinal tracts of animals, sewage sludge, the deep subsurface of Earth, and many other environments. In these anoxic habitats, microorganisms, particularly prokaryotes, thrive.

#### 3.6.5.1- Oxygen Classes of Microorganisms

Microorganisms vary in their need for, or tolerance of, oxygen. In fact, microorganisms can be grouped according to how oxygen affects them. Aerobes can grow at full oxygen tensions (air is 21%  $O_2$ ) and respire oxygen in their metabolism. Many aerobes can even tolerate elevated concentrations of oxygen (hyperbaric oxygen). Microaerophiles, by contrast, are aerobes that can use oxygen only when it is present at levels reduced from that in air (microoxic conditions). This is because of their limited capacity to respire or because they contain some oxygen-

sensitive molecule such as an oxygen-labile enzyme. Many aerobes are **facultative**, meaning that under the appropriate nutrient and culture conditions they can grow under either oxic or anoxic conditions.

Some organisms cannot respire oxygen; such organisms are called **anaerobes**. There are two kinds of anaerobes: **aerotolerant anaerobes**, which can tolerate oxygen and grow in its presence even though they cannot use it, and **obligate anaerobes**, which are inhibited or even killed by O<sub>2</sub>. The reason obligate anaerobes are killed by oxygen is unknown, but it is likely because they are unable to detoxify some of the products of oxygen metabolism.

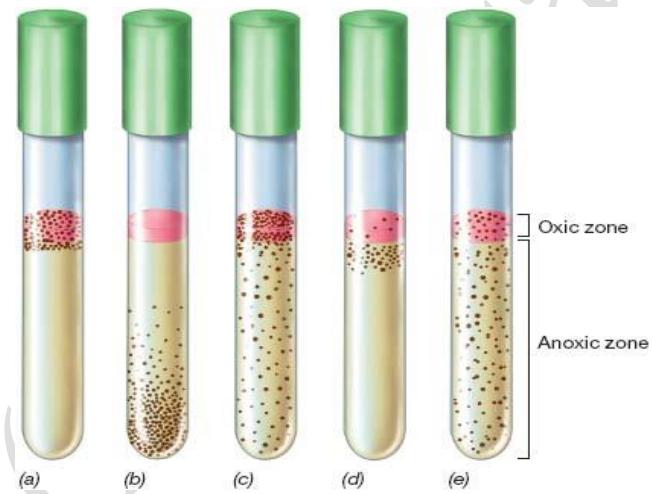
So far as is known, obligate anaerobiosis is found in only three groups of microorganisms: a wide variety of prokaryotes, a few *fungi*, and a few *protozoa*. The best-known group of *obligately anaerobic Bacteria* belongs to the genus *Clostridium*, a group of gram-positive endospore-forming rods. *Clostridia* are widespread in soil, lake sediments, and intestinal tracts and are often responsible for spoilage of canned foods. Other obligately anaerobic organisms are the methanogens and many other *Archaea*, the *sulfate-reducing* and *homoacetogenic bacteria*, and many of the *bacteria* that inhabit the animal gut and oral cavity. Among obligate anaerobes, however, the sensitivity to oxygen varies greatly. Some species can tolerate traces of oxygen or even full exposure to oxygen, whereas others cannot.

### **3.6.5.2- Culture Techniques for *Aerobes* and *Anaerobes***

For the growth of many *aerobes*, it is necessary to provide extensive aeration. This is because the oxygen that is consumed by the organisms during growth is not replaced fast enough by simple diffusion

from the air. Therefore, forced aeration of cultures is needed and can be achieved by either vigorously shaking the flask or tube on a shaker or by bubbling sterilized air into the medium through a fine glass tube or porous glass disc. *Aerobes* typically grow better with forced aeration than with oxygen supplied from simple diffusion.

For the culture of *anaerobes*, the problem is not to provide air, but to exclude it. *Obligate anaerobes* vary in their sensitivity to oxygen, and procedures are available for reducing the oxygen content of cultures. Some of these techniques are simple and suitable mainly for less oxygen-sensitive organisms; others are more complex, but necessary for growth of *obligate anaerobes*. Bottles or tubes filled completely to the top with culture medium and provided with tightly fitting stoppers provide suitably anoxic conditions for organisms that are not overly sensitive to small amounts of oxygen. A chemical called a reducing agent may be added to culture media; the reducing agent reacts with oxygen and reduces it to H<sub>2</sub>O. An example is thioglycolate, which is added to thioglycolate broth, a medium commonly used to test an organism's requirements for oxygen (Figure 7).



**Figure 7: Growth versus oxygen concentration.** (a–e) Aerobic, anaerobic, facultative, microaerophilic, and aerotolerant anaerobe growth, as revealed by the position of microbial colonies (depicted here as black dots) within tubes of thioglycolate broth culture medium. A small amount of agar has been added to keep the liquid from becoming disturbed, and the redox dye, resazurin, which is pink when oxidized and colorless when reduced, is added as a redox indicator. (a) Oxygen penetrates only a short distance into the tube, so *obligate aerobes* only grow close to the surface. (b) *Anaerobes*, being sensitive to oxygen, grow only away from the surface. (c) *Facultative aerobes* are able to grow in either the presence or the absence of oxygen and thus grow throughout the tube. However, growth is better near the surface because these organisms can respire. (d) *Microaerophiles* grow away from the most oxic zone. (e) *Aerotolerant anaerobes* grow throughout the tube. Growth is not better near the surface because these organisms can only ferment.

# **Chapter 4**

## **Bacterial Classification & Identification**

## Classification and Identification of Bacteria

The classification and identification of organisms are two separate but inter-related processes. **Classification** involves the identification of groups of organism that share common properties and that differ from other groups. **Identification** entails the assignation of an unknown organism to a group within a scheme of classification.

For more than a century bacteria have been classified according to their "Gram reaction" named after Christian Gram who devised the protocol for his staining process in 1884. This is based upon their ability or otherwise to retain the crystal violet-iodine complex when treated with organic solvents such as acetone or alcohol. Gram-positive bacteria retain the stain, and hence appear purple or blue-black when visualized by bright-field microscopy. In contrast, Gram-negative bacteria cannot retain the dye complex, and need to be counterstained with a red dye such as carbol fuchsin before they can be seen in the bright-field microscope.

As well as using their Gram reaction, bacteriologists also use the shape of bacteria to classify them. Bacteria display three basic shapes: round (*cocci*, from the Greek *kokkos* - a berry), rod shaped (*bacilli*, from the Latin *bacillus* - a stick or rod), or *spiral*.

**Until recently classification has done on the basis of such traits as:**

**1. Shape**

- *Bacilli*: rod-shaped
- *Cocci*: spherical
- *Spirilla*: curved walls

**2. Ability to form spores.**

**3. Method of energy production** (glycolysis for *anaerobes*, cellular respiration for *aerobes*).

**4. Nutritional requirements.**

**5. Reaction to the Gram stain.**

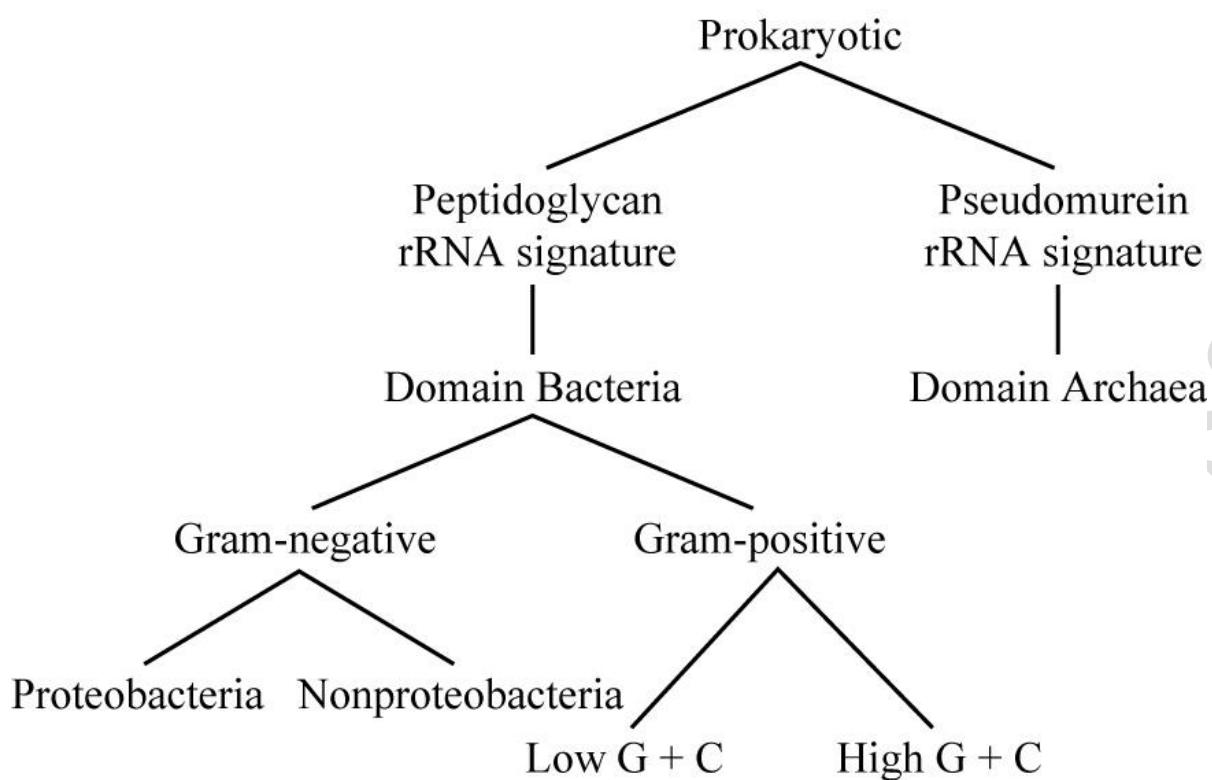
6. More recently, **genome sequencing**, especially of their **16S ribosomal RNA (rRNA)**, has provided additional insights into the evolutionary relationships among the bacteria.



Gram-positive bacteria are encased in a plasma membrane covered with a thick wall of peptidoglycan. Gram-negative bacteria are encased in a triple-layer. The outermost layer contains lipopolysaccharide (LPS).

Although the Gram stain might seem an arbitrary criterion to use in bacterial taxonomy, it does, in fact, distinguish between two fundamentally different kinds of bacterial cell walls and reflects a natural division among the bacteria.

- **Bergey's Manual of Systematic Bacteriology**
  - Classifies bacteria via evolutionary or genetic relationships.
- **Bergey's Manual of Determinative Bacteriology**
  - Classifies bacteria by cell wall composition, morphology, biochemical tests, differential staining, etc.



### Domain: Bacteria

#### A- Phylum: *Proteobacteria*

- Contains most of the gram negative bacteria.
- Relationships have been put together via Ribosomal RNA studies.
- Split up into five classes. *Alpha, beta, gamma, delta, and epsilon*.

#### 1- *Alphaproteobacteria*

The *Alphaproteobacteria* contain many bacteria that are capable of nitrogen fixation in symbiosis with plants.

- ***Rhizobium***: Fix nitrogen in the roots of plants.

Also includes several plant and human pathogens.

#### a- Human pathogens

- ***Bartonella***
  - ***B. hensela***: Cat-scratch disease
- ***Brucella***: Brucellosis
- ***Rickettsia***: Arthropod-borne, spotted fevers

### b- Plant pathogen

- *Agrobacterium*: Insert a plasmid into plant cells, inducing a tumor.

## 2- The *Betaproteobacteria*

- *Neisseria*

- Usually inhabit the mucous membranes of mammals.
- *N. meningitidis*
- *N. gonorrhoeae*

## 3- The *Gammaproteobacteria*

- *Pseudomonas*

- Opportunistic pathogens
- Metabolically diverse
- Polar flagella

- *Legionella*

- Found in streams, warm-water pipes, cooling towers
- *Legionellosis* or legionaire's disease

- Enteric Bacterial Genera

- *Escherichia*-Very commonly found in human intestines. Known for its ability to cause very serious food-borne disease.
- *Salmonella*-Almost all members of this genera are potential pathogens.
- *Serratia*- Can be found on catheters, in saline irrigation solutions, and in other supposedly sterile situations.
- *Proteus*-very motile genera, many flagella, can cause UTI's (Urinary Tract infection) or wound infections.

#### **4- The *Epsilonproteobacteria***

- ***Helicobacter***

- Multiple flagella
- Peptic ulcers
- Stomach cancer

#### **B- Phylum: *Cyanobacteria***

- Oxygenic photosynthesis
- Heterocysts-specialized cells that fix nitrogen.

#### **C- Phylum: *Firmicutes***

Comparison of their sequenced genomes reveals that all the Gram-positive *rods* and *cocci* as well as the *mycoplasmas* belong to a single clade that has been named the ***Firmicutes***.

- Low G + C ratio (Guanine and cytosine bases in DNA).
- Gram-positive bacteria.

##### **1- *Clostridiales***

- ***Clostridium***

- Endospore-producing
- Obligate anaerobes

*C. tetani*- tetanus,

*C. Botulinum*- Botulism

*C. perfringens*- gas gangrene,

*C. difficile*- serious diarrhea, especially when undergoing antibiotic therapy.

##### **2- *Bacillales***

- ***Bacillus***

- Endospore-producing rods
- *B. anthracis* –cause anthrax
- *Staphylococcus*
  - *S. aureus*-cause a lot of hospital infection.
- *Streptococcus*-

Members of this genus are probably responsible for more diseases than any other group of bacteria. Pneumonia, strep throat, cavities and scarlet fever.

#### D- Phylum: *Actinobacteria*

- High G + C (guanine and cytosine levels in DNA).
- Gram-positive

#### Important Genera

- *Mycobacterium*
  - *M. tuberculosis*
  - *M. leprae*-causes leprosy.
- *Propionibacterium*-Some species help make Swiss cheese, others cause acne.
- *Streptomyces*-produce a large number of antibiotics.

#### E- Phylum: *Spirochaetes*

- *Treponema*
  - *T. pallidum* is the cause of syphilis.