BACTERIOLOGY

FOR 2d YEAR STUDENTS

MICROBIOLOGY/CHEM & MICROBIOLOGY

FACULTY OF SCIENCE

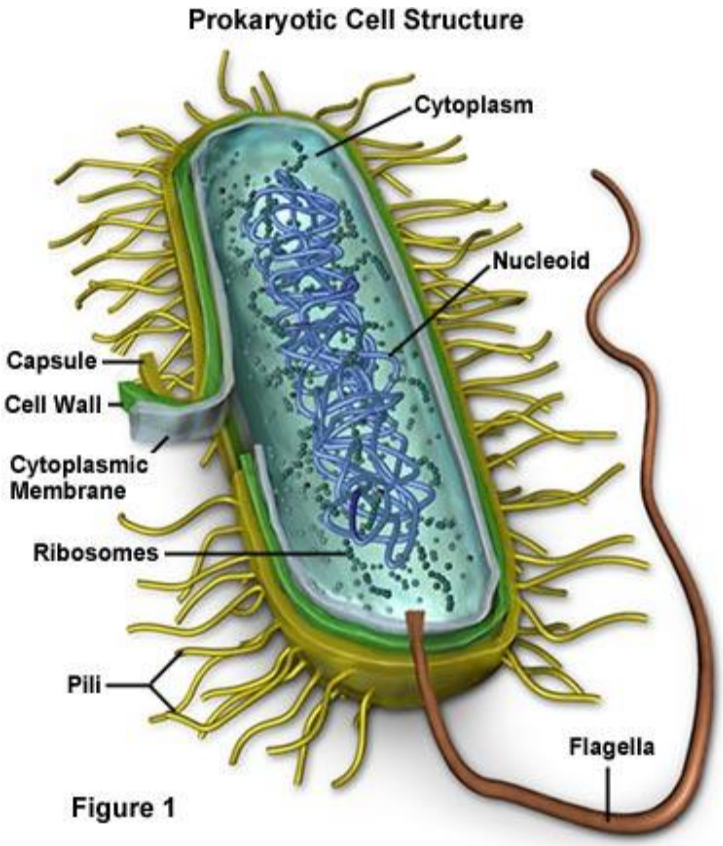


Figure 1

PROF. DR. WESAM M. A. SALEM

COD: MIC 201
2022

اسم المقرر: علم البكتريا (الرمز الكودي:Mic201

أهداف المقرر ونواتج التعلم المستهدفة

أهداف المقرر: فهم ودراسة أساسيات النمو للبكتريا والكشف عن النشاط الحيوى وطرق الفحص للعينات البكتيرية

التعرف على طرق العزل والتعريف المبدئى وطرق التعامل مع البكتريا فى بعض التطبيقات وطرق حل المشكلات المرتبطة بها فى مجالات الحياة المختلفة.

نواتج التعلم المستهدفة

1- المعلومات والمفاهيم:

- **يصف الطالب تركيب الخلية البكتيرية ويتعرف على وضع الكائنات الأولية بالنسبة لباقى الكائنات ويسمى وظائف المحتويات الخلوية والأغلفة**
- **يحدد الطالب مراحل النمو المختلفة للبكتريا (منحنى النمو) والظروف التى تتحكم فيه وخاصة التعقيم وأنواع مثبطات النمو المختلفة والمضادات الحيوية وطرق قياس النمو**
- **يذكر الطالب دور الأنواع البكتيرية المختلفة فى المحافظة على العناصر فى الطبيعة ويتعرف على صفات بعض المجموعات الهامة ودور البكتريا فى التطبيقات المختلفة والأمراض وتفادى أضرارها**

2- المهارات الذهنية:

- **يميز الطالب بين الأنواع الهامة للبكتريا ودورها فى البيئات المختلفة**
- **يربط الطالب بين نظم نقل الجزيئات من والى الخلية وميكانيكية عملها وحاجتها للطاقة**
- **يوضح العناصر الرئيسية للتحكم فى النمو والأيض الخلوى وطرق قياسه باستخدام تقنيات مختلفة**
- **يحلل بعض التطبيقات الهامة ودور البكتريا فيها وكيفية توفير الظروف المثلى**
- **يقارن بين البكتريا الضارة (المسببة للأمراض الشائعة وفساد الأغذية- مع طرق حفظها) وبين الأنواع الأخرى النافعة**
-

3- المهارات المهنية:

- **يستخدم الميكروسكوب الضوئى ووسائل وأدوات التعقيم المختلفة**
- **يتناول الأشكال الرئيسية للبكتريا تحت الميكروسكوب وبعض التركيبات الخلوية الداخلية**
- **يستخدم طرق تقدير نمو البكتريا وبعض التفاعلات الحيوية كدليل على النمو وللتفرقة بين الأنواع**
- **يتناول تأثير مضادات النمو الفيزيائية والكيميائية على البكتريا وخاصة المضادات الحيوية**
- **يستخدم الأدوات المعملية المناسبة لقياس تأثير بعض المواد الأخرى على الخلية**
- **يستخدم طرق عزل البكتريا من البيئات المختلفة ويجرى خطوات التعريف والتحكم فى النمو بالتحفيز أو التثبيط**

4- المهارات العامة:

- **المناقشة واستحضار المعلومات الأساسية خلال المحاضرات والدروس العملية**
- **التعامل المناسب والتواصل والتفاعل بالعمل الجماعى والمشاركة.**

OVERVIEW OF BACTERIOLOGY

The Scope of Bacteriology

The Bacteria are a group of single-cell microorganisms with **procaryotic** cellular configuration. The genetic material (DNA) of procaryotic cells exists unbound in the cytoplasm of the cells. There is **no nuclear membrane**, which is the definitive characteristic of eucaryotic cells such as those that make up plants and animals. Until recently, bacteria were the only known type of procaryotic cell, and the discipline of biology related to their study is called **bacteriology**. In the 1980's, with the outbreak of molecular techniques applied to phylogeny of life, another group of procaryotes was defined and informally named "archaebacteria". This group of procaryotes has since been renamed **Archaea.** The current science of bacteriology includes the study of both domains of procaryotic cells. but the name "bacteriology" is not likely to change to reflect the inclusion of archaea in the discipline.

The Origin of Life

When life arose on Earth about 4 billion years ago, the first types of cells to evolve were procaryotic cells. For approximately 2 billion years, procaryotic-type cells were the only form of life on Earth. The oldest known sedimentary rocks, from Greenland, are about 3.8 billion years old. The oldest known fossils are procaryotic cells, 3.5 billion years in age, found in Western Australia and South Africa. The nature of these fossils, and the chemical composition of the rocks in which they are found, indicate that **lithotrophic** and **fermentative** modes of metabolism were the first to evolve in early procaryotes. **Photosynthesis** developed in bacteria a bit later, at least 3 billion years ago. **Anoxygenic photosynthesis** (bacterial photosynthesis, which is anaerobic and does not produce O_2) preceded **oxygenic photosynthesis** (plant-type photosynthesis, which yields O_2). However, oxygenic photosynthesis also arose in procaryotes, specifically in the cyanobacteria, which existed millions of years before the evolution of green algae plants. Larger, more complicated eucaryotic cells did not appear until much later, between 1.5 and 2 billion years ago.

Figure 1. Opalescent Pool in Yellowstone National Park, Wyoming USA. Conditions for life in this environment are similar to Earth over 2 billion years ago. In these types of hot springs, the orange, yellow and brown colors are due to pigmented photosynthetic bacteria which make up the microbial mats. The mats are literally teeming with bacteria. Some of these bacteria such as *Synechococcus* conduct oxygenic photosynthesis, while others such as *Chloroflexus* conduct anoxygenic photosynthesis. Other non-photosynthetic bacteria, as well as thermophilic and acidophilic Archaea, are also residents of the hot spring community.

The archaea and bacteria differ fundamentally in their structure from eucaryotic cells, which always contain a membrane-enclosed nucleus, multiple chromosomes, and various other membranous organelles such as mitochondria, chloroplasts, the golgi apparatus, vacuoles, etc. Unlike plants and animals, archaea and bacteria are unicellular organisms that do not develop or differentiate into multicellular forms. Some bacteria grow in filaments or masses of cells, but each cell in the colony is identical and capable of independent existence. The cells may be adjacent to one another because they did not separate after cell division or because they remained enclosed in a common sheath or slime secreted by the cells, but typically there is no continuity or communication between the cells.

Size and Distribution of Bacteria and Archaea

Most procaryotic cells are very small compared to eucaryotic cells. A typical bacterial cell is about 1 micrometer in diameter or width, while most eucaryotic cells are from 10 to 100 micrometers in diameter. Eucaryotic cells have a much greater volume of cytoplasm and a much lower surface: volume ratio than procaryotic cells. A typical procaryotic cell is about the size of a eucaryotic mitochondrion. Since procaryotes are too small to be seen except with the aid of a microscope, it is usually not appreciated that they are the most abundant form of life on the planet, both in terms of biomass and total numbers of species. For example, in the sea, procaryotes make up 90

percent of the total combined weight of all organisms. In a single gram of fertile agricultural soil there may be in excess of 10^9 bacterial cells, outnumbering all eucaryotic cells there by 10,000: 1. About 3,000 distinct species of bacteria and archaea are recognized, but this number is probably less than one percent of all the species in nature. These unknown procaryotes, far in excess of undiscovered or unstudied plants, are a tremendous reserve of genetic material and genetic information in nature that awaits exploitation.

Procaryotes are found in all of the habitats where eucaryotes live, but, as well, in many natural environments considered too extreme or inhospitable for eucaryotic cells. Thus, the outer limits of life on Earth (hottest, coldest, driest, etc.) are usually defined by the existence of procaryotes. Where eucaryotes and procaryotes live together, there may be mutualistic associations between the organisms that allow both to survive or flourish. The organelles of eucaryotes (mitochondria and chloroplasts) are thought to be remnants of Bacteria that invaded, or were captured by, primitive eucaryotes in the evolutionary past. Numerous types of eucaryotic cells that exist today are inhabited by endosymbiotic procaryotes.

From a metabolic standpoint, the procaryotes are extraordinarily diverse, and they exhibit several types of metabolism that are rarely or never seen in eucaryotes. For example, the biological processes of **nitrogen fixation** (conversion of atmospheric nitrogen gas to ammonia) and **methanogenesis** (production of methane) are metabolically-unique to procaryotes and have an enormous impact on the nitrogen and carbon cycles in nature. Unique mechanisms for energy production and photosynthesis are also seen among the Archaea and Bacteria.

The lives of plants and animals are dependent upon the activities of bacterial cells. Bacteria enter into various types of symbiotic relationships with plants and animals that usually benefit both organisms, although a few bacteria are agents of disease.

The metabolic activities of procaryotes in soil habitats have an enormous impact on soil fertility that can affect agricultural practices and crop yields. In the global environment, procaryotes are absolutely essential to drive the cycles of elements that

make up living systems, i.e., the carbon, oxygen, nitrogen and sulfur cycles. The origins of the plant cell chloroplast and plant-type (oxygenic) photosynthesis are found in procaryotes. Most of the earth's atmospheric oxygen may have been produced by free-living bacterial cells. The bacteria fix nitrogen and a substantial amount of CO_2, as well.

Bacteria or bacterial products (including their genes) can be used to increase crop yield or plant resistance to disease, or to cure or prevent plant disease. Bacterial products include antibiotics to fight infectious disease, as well as components for vaccines used to prevent infectious disease. Because of their simplicity and our relative understanding of their biological processes, the bacteria provide convenient laboratory models for study of the molecular biology, genetics, and physiology of all types of cells, including plant and animal cells.

TAXONOMY AND CLASSIFICATION OF PROCARYOTES

Haeckel (1866) was the first to create a natural Kingdom for the microorganisms, which had been discovered nearly two centuries before by van Leeuwenhoek. He placed all unicellular (microscopic) organisms in a new kingdom, **"Protista"**, separated from plants (**Plantae**) and animals (**Animalia**), which were multicellular (macroscopic) organisms. The development of the electron microscope in the 1950's revealed a fundamental dichotomy among Haeckel's **"Protista"**: some cells contained a membrane-enclosed nucleus, and some cells lacked this intracellular structure. The latter were temporarily shifted to a fourth kingdom, **Monera** (or **Moneres**), the procaryotes (also called **Procaryotae**). **Protista** remained as a kingdom of unicellular eucaryotic microorganisms. Whittaker refined the system into five kingdoms in 1967, by identifying the **Fungi** as a separate multicellular eucaryotic kingdom of organisms, distinguished by their absorptive mode of heterotrophic nutrition.

In the 1980's, Woese began phylogenetic analysis of all forms of cellular life based on comparative sequencing of the small subunit ribosomal RNA (ssrRNA) that is contained in all organisms. A new dichotomy was revealed, this time among the procaryotes: there existed two types of procaryotes, as fundamentally unrelated to

one another as they are to eucaryotes. Thus, Woese defined the **three cellular Domains of life** as **Eucarya**, **Bacteria** and **Archaea**. Whittaker's Plant, Animal and Fungi kingdoms (all of the multicellular eucaryotes) are at the end of a very small branch of the tree of life, and all other branches lead to microorganisms, either procaryotes (Bacteria and Archaea), or protists (unicellular algae and protozoa), thus establishing clearly that microbial life is the predominant form of life on the planet.

Although the definitive difference between Woese's **Archaea** and **Bacteria** is based on fundamental differences in the nucleotide base sequence in the 16S ribosomal RNA, there are many biochemical and phenotypic differences between the two groups of procaryotes. (Table 1). The phylogenetic tree indicates that **Archaea** are more closely related to **Eucarya** than are **Bacteria**. This relatedness seems most evident in the similarities between transcription and translation in the **Archaea** and the **Eucarya**. However, it is also evident that the **Bacteria** have evolved into chloroplasts and mitochondria, so that these eucaryotic organelles derive their lineage from this group of procaryotes. Perhaps the biological success of eucaryotic cells springs from the evolutionary merger of the two procaryotic life forms.

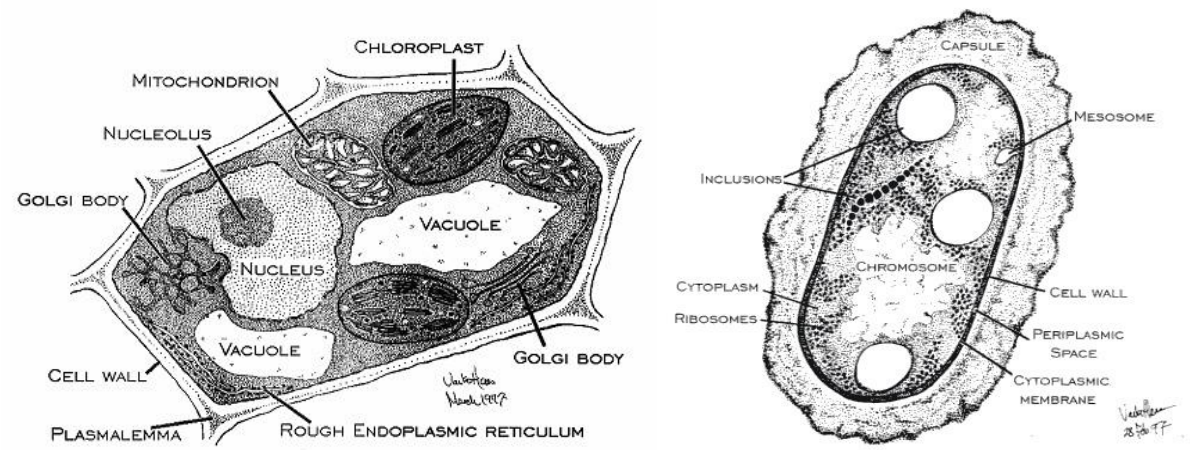


Figure 2 (Right) The structure of a typical procaryotic cell, in this case, a Gram-negative bacterium, compared with (Left) a typical eucaryotic cell (plant cell). The procaryote is about 1 micrometer in diameter and about the size of the eucaryotic chloroplast or mitochondrion.

Table 1. Phenotypic properties of Bacteria and Archaea compared with Eucarya.

Property	Biological Domain		
	Eucarya	Bacteria	Archaea
Cell configuration	eucaryotic	Prokaryotic	Prokaryotic
Nuclear membrane	present	Absent	Absent
Number of chromosomes	>1	1	1
Chromosome topology	linear	Circular	circular
Murein in cell wall	-	+	-
Cell membrane lipids	ester-linked glycerides; unbranched; polyunsaturated	ester-linked glycerides; unbranched; saturated or monounsaturated	ether-linked branched; saturated
Cell membrane sterols	present	Absent	Absent
Organelles (mitochondria and chloroplasts)	present	Absent	Absent
Ribosome size	80S (cytoplasmic)	70S	70S
Cytoplasmic streaming	+	-	-
Meiosis and mitosis	present	Absent	Absent
Transcription and translation coupled	-	+	+
Amino acid initiating protein synthesis	methionine	N-formyl methionine	methionine
Protein synthesis inhibited by streptomycin and chloramphenicol	-	+	-
Protein synthesis inhibited by diphtheria toxin	+	-	+

IDENTIFICATION OF BACTERIA

The criteria used for microscopic identification of procaryotes include cell shape and grouping, Gram-stain reaction, and motility. Bacterial cells almost invariably take one of three forms: rod (**bacillus**), sphere (**coccus**), or spiral (**spirilla** and **spirochetes**). Rods that are curved are called **vibrios**. Fixed bacterial cells stain either Gram-positive (purple) or Gram-negative (pink); motility is easily determined by observing living specimens. Bacilli may occur singly or form chains of cells; cocci may form chains (**streptococci**) or grape-like clusters (**staphylococci**); spiral shape cells are almost always motile; cocci are almost never motile. This

nomenclature ignores the **actinomycetes**, a prominent group of branched bacteria which occur in the soil. But they are easily recognized by their colonies and their microscopic appearance.

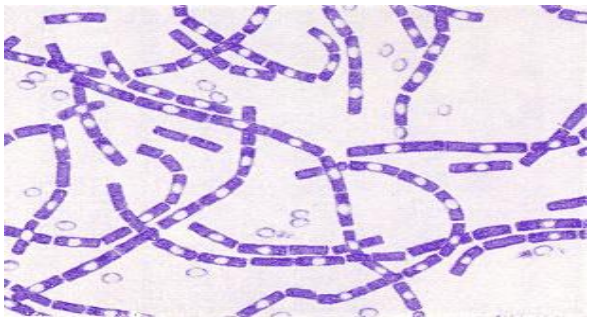


Figure 3. Gram stain of *Bacillus anthracis, the* cause of anthrax.

Such easily-made microscopic observations, combined with knowing the natural environment of the organism, are important aids to identify the group, if not the exact genus, of a bacterium - providing, of course, that one has an effective key. Such a key is **Bergey's Manual of Determinative Bacteriology**, the "field guide" to identification of the bacteria. Bergey's Manual describes affiliated groups of **Bacteria** and **Archaea** based on a few easily observed microscopic and physiologic characteristics. Further identification requires biochemical tests which will distinguish genera among families and species among genera. Strains within a single species are usually distinguished by genetic or immunological criteria.

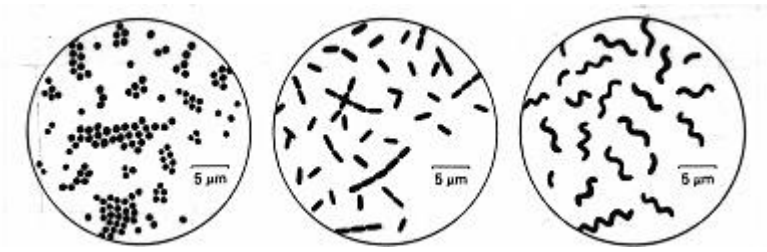


Figure 4. Size and fundamental shapes of procaryotes revealed by three genera of Bacteria (l to r): *Staphylococcus* (spheres), *Lactobacillus* (rods), and *Aquaspirillum* (spirals).

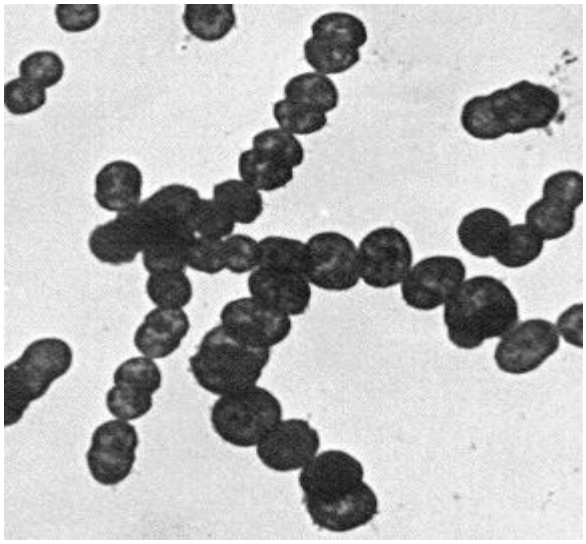


Figure 5. Chains of dividing streptococci. Electron micrograph of *Streptococcus pyogenes*.

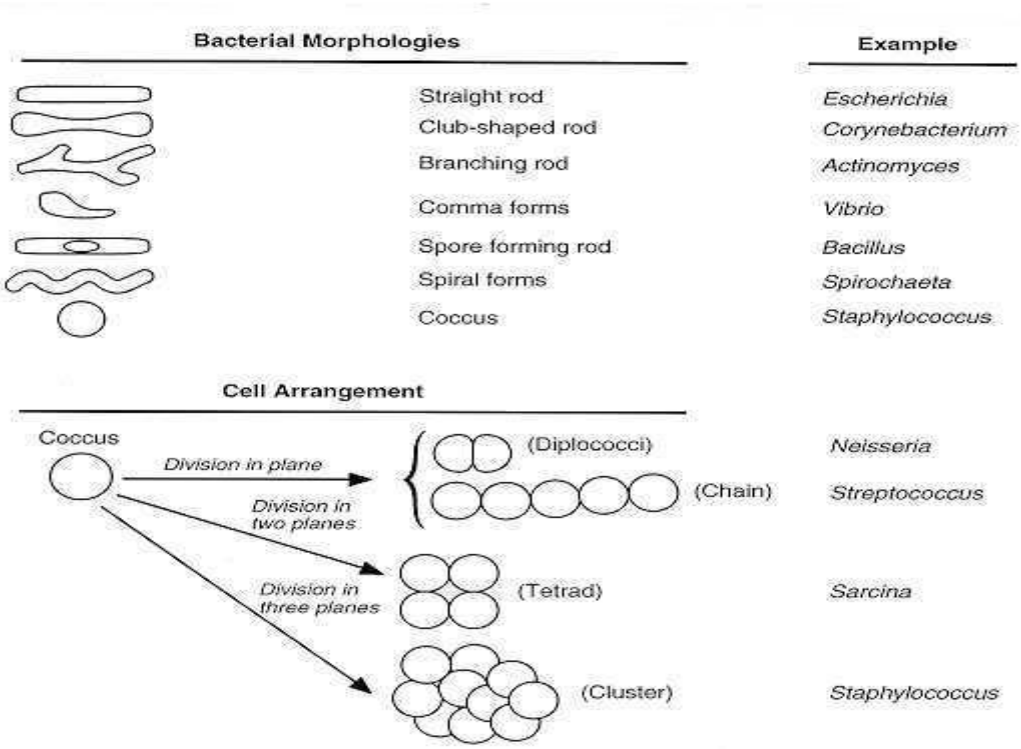


Figure 6. Different shapes and arrangements of bacterial cells, with examples.

STRUCTURE AND FUNCTION OF PROCARYOTIC CELLS

Procaryotes are unicellular organisms of relatively simple construction, especially if compared to eukaryotes. Whereas eukaryotic cells have a preponderance of organelles with separate cellular functions, procaryotes carry out all cellular functions

as individual units. A procaryotic cell has five essential structural components: a **genome (DNA)**, **ribosomes**, **cell membrane**, **cell wall**, and some sort of **surface layer** which may or may not be an inherent part of the wall. Other than enzymatic reactions, all the cellular reactions incidental to life can be traced back to the activities of these macromolecular structural components. Thus, functional aspects of procaryotic cells are related directly to the structure and organization of the macromolecules in their cell make-up, i.e., DNA, RNA, phospholipds, proteins and polysaccharides. Diversity within the primary structure of these molecules accounts for the diversity that exists among procaryotes.

At one time it was thought that bacteria were essentially "bags of enzymes" with no inherent cellular architecture. The development of the electron microscope, in the 1950s, revealed the distinct anatomical features of bacteria and confirmed the suspicion that they lacked a nuclear membrane. Structurally, a procaryotic cell (Figure 1) has three architectural regions: **appendages** (attachments to the cell surface) in the form of **flagella** and **pili (or fimbriae)**; a **cell envelope** consisting of a **capsule**, **cell wall** and **plasma membrane**; and a **cytoplasmic region** that contains the cell **genome** (**DNA**) and **ribosomes** and various sorts of **inclusions**. In this chapter, we will discuss the anatomical structures of procaryotic cells in relation to their adaptation, function and behavior in natural environments.

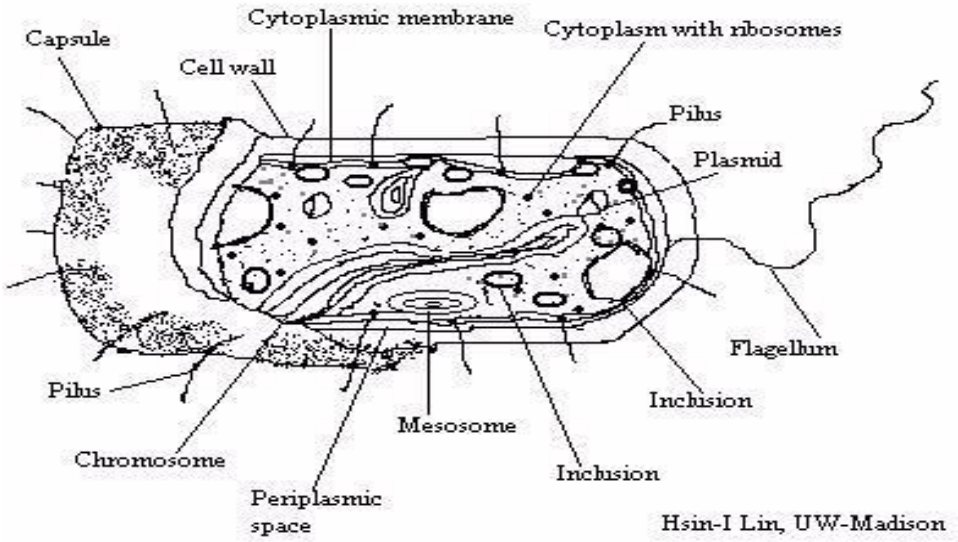


Figure 1. Schematic drawing of a typical bacterium.

Table 1. Summary: Characteristics of typical bacterial cell structures.

Structure	Function(s)	Predominant chemical composition
Flagella	Swimming movement	Protein
Pili		
Sex pilus	Mediates DNA transfer during conjugation	Protein
Common pili or fimbriae	Attachment to surfaces; protection against phagotrophic engulfment	Protein
Capsules (includes "slime layers" and glycocalyx)	Attachment to surfaces; protection against phagocytic engulfment, occasionally killing or digestion; reserve of nutrients or protection against desiccation	Usually polysaccharide; occasionally polypeptide
Cell wall		
Gram-positive bacteria	Prevents osmotic lysis of cell protoplast and confers rigidity and shape on cells	Peptidoglycan (murein) complexed with teichoic acids
Gram-negative bacteria	Peptidoglycan prevents osmotic lysis and confers rigidity and shape; outer membrane is permeability barrier; associated LPS and proteins have various functions	Peptidoglycan (murein) surrounded by phospholipid protein-lipopolysaccharide "outer membrane"
Plasma membrane	Permeability barrier; transport of solutes; energy generation; location of numerous enzyme systems	Phospholipid and protein
Ribosomes	Sites of translation (protein synthesis)	RNA and protein
Inclusions	Often reserves of nutrients; additional specialized functions	Highly variable; carbohydrate, lipid, protein or inorganic
Chromosome	Genetic material of cell	DNA
Plasmid	Extrachromosomal genetic material	DNA

Appendages

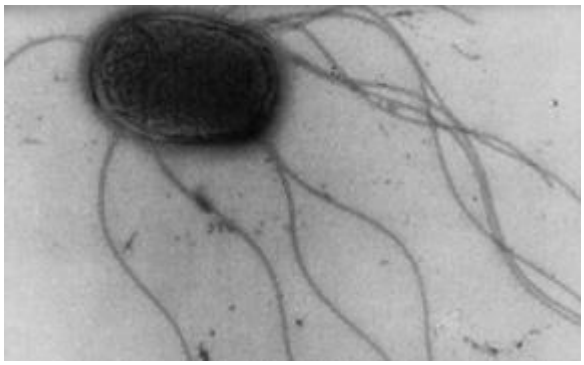


Figure 2. *Salmonella enteritidis* TEM about 10,000X. *Salmonella* is an enteric bacterium related to *E. coli.* The enterics are motile by means of peritriochous flagella.

Flagella

Flagella are filamentous protein structures attached to the cell surface that provide the swimming movement for most motile procaryotes. Procaryotic flagella are much thinner than eukaryotic flagella. The diameter of a procaryotic flagellum is about 20 nanometers, well-below the resolving power of the light microscope. The flagellar filament is rotated by a motor apparatus in the plasma membrane allowing the cell to swim in fluid environments. Bacterial flagella are powered by proton motive force (chemiosmotic potential) established on the bacterial membrane, rather than ATP hydrolysis which powers eukaryotic flagella. About half of the bacilli and all of the spiral and curved bacteria are motile by means of flagella. Very few cocci are motile, which reflects their adaptation to dry environments and their lack of hydrodynamic design.

The ultrastructure of the flagellum of *E. coli* is illustrated in (Figure 3) below. About 50 genes are required for flagellar synthesis and function. The flagellar apparatus consists of several **distinct proteins**: a system of **rings** embedded in the cell envelope (the **basal body**), a **hook-like structure** near the cell surface, and the **flagellar filament**. The innermost rings, the **M** and **S** rings, located in the plasma membrane, comprise the motor apparatus. The outermost rings, the **P** and **L** rings, located in the periplasm and the outer membrane respectively, function as bushings to support the rod where it is joined to the hook of the filament on the cell surface.

As the **M** ring turns, powered by an influx of protons, the rotary motion is transferred to the filament which turns to propel the bacterium.

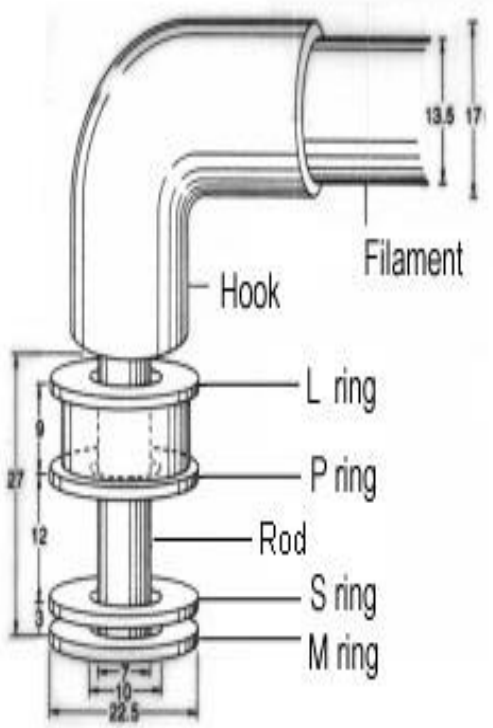


Figure 3. The ultrastructure of a bacterial flagellum. Measurements are in nanometers. The flagellum of *E. coli* consists of three parts, filament, hook and basal body, all composed of different proteins. The basal body and hook anchor the whip-like filament to the cell surface. The basal body consists of four ring-shaped proteins stacked like donuts around a central rod in the cell envelope. The inner rings, associated with the plasma membrane, are the flagellar powerhouse for activating the filament. The outer rings in the peptidoglycan and outer membrane are support rings or "bushings" for the rod. The filament rotates and contracts which propels and steers the cell during movement. Compare with Figure 21 below.

Flagella may be variously distributed over the surface of bacterial cells in distinguishing patterns, but basically flagella are either **polar** (one or more flagella arising from one or both poles of the cell) or **peritrichous** (lateral flagella distributed over the entire cell surface). Flagellar distribution is a genetically-distinct trait that is occasionally used to characterize or distinguish bacteria. For example, among Gram-negative rods, pseudomonads have polar flagella to distinguish them from enteric bacteria, which have peritrichous flagella.

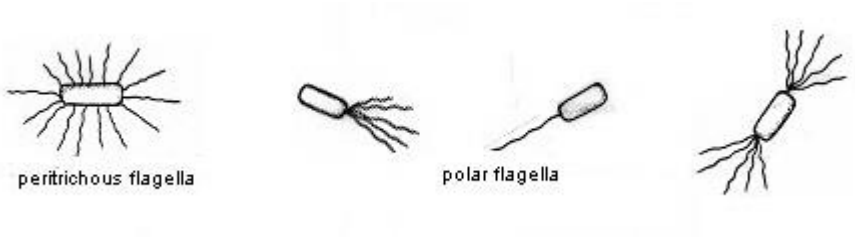


Figure 4. Different arrangements of bacterial flagella. Swimming motility, powered by flagella, occurs in half the bacilli and most of the spirilla. Flagellar arrangements, which can be determined by staining and microscopic observation, may be a clue to the identity of a bacterium. See Figure 5 below.

Flagella were proven to be organelles of bacterial motility by shearing them off (by mixing cells in a blender) and observing that the cells could no longer swim although they remained viable. As the flagella were regrown and reached a critical length, swimming movement was restored to the cells. The flagellar filament grows at its tip (by the deposition of new protein subunits) not at its base (like a hair).

Procaryotes are known to exhibit a variety of types of **tactic behavior**, i.e., the ability to move (swim) in response to environmental stimuli. For example, during **chemotaxis** a bacterium can sense the quality and quantity of certain chemicals in its environment and swim towards them (if they are useful nutrients) or away from them (if they are harmful substances). Other types of tactic response in procaryotes include **phototaxis**, **aerotaxis** and **magnetotaxis**. The occurrence of tactic behavior provides evidence for the ecological (survival) advantage of flagella in bacteria and other procaryotes.

Detecting Bacterial Motility

Since motility is a primary criterion for the diagnosis and identification of bacteria, several techniques have been developed to demonstrate bacterial motility, directly or indirectly.

1. **Flagellar stains** outline flagella and show their pattern of distribution. If a bacterium possesses flagella, it is presumed to be motile.

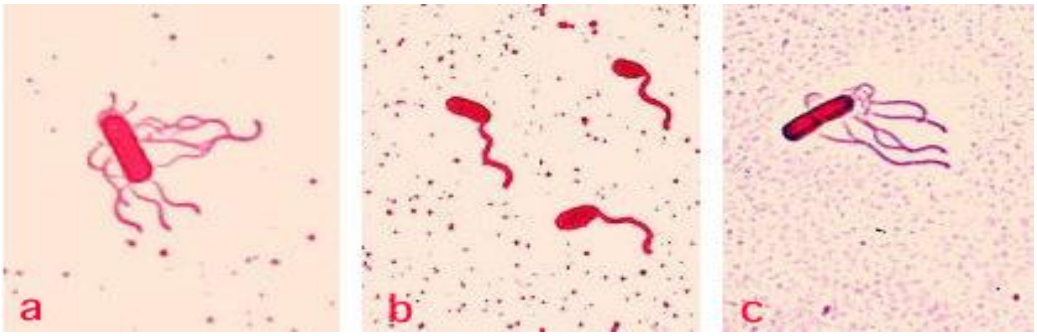


Figure 5. Flagellar stains of three bacteria a. *Bacillus cereus* b. *Vibrio cholerae* c. *Bacillus brevis*. Since the bacterial flagellum is below the resolving power of the light microscope, although bacteria can be seen swimming in a microscope field, the organelles of movenent cannot be detected. Staining techniques such as Leifson's method utilize dyes and other components that precipitate along the protein filament and hence increase its effective diameter. Flagellar distribution is occasionally used to differentiate between morphologically related bacteria. For example, among the Gram-negative motile rod-shaped bacteria, the enterics have peritrichous flagella while the pseudomonads have polar flagella.

2. **Motility test medium** demonstrates if cells can swim in a semisolid medium. A semisolid medium is inoculated with the bacteria in a straight-line stab with a needle. After incubation, if turbidity (cloudiness) due to bacterial growth can be observed away from the line of the stab, it is evidence that the bacteria were able to swim through the medium.

OFF THE WALL. Julius Adler exploited this observation during his studies of chemotaxis in *E. coli.* He prepared a gradient of glucose by allowing the sugar to diffuse into a semisolid medium from a central point in the medium. This established a concentration gradient of glucose along the radius of diffusion. When *E. coli* cells were seeded in the medium at the lowest concentration of glucose (along the edge of the circle), they swam up the gradient towards a higher concentration (the center of the circle), exhibiting their chemotactic response to swim towards a useful nutrient. Later, Adler developed a tracking microscope that could record and film the track that *E. coli* takes as it swims towards a chemotactic attractant or away from a chemotactic repellent. This led to an understanding of the mechanisms of bacterial chemotaxis, first at a structural level, then at a biomolecular level.

3. **Direct microscopic observation** of living bacteria in a wet mount. One must look for transient movement of swimming bacteria. Most uincellular bacteria, because of their small size, will shake back and forth in a wet mount observed at 400X or 1000X. This is Brownian movement, due to random collisions between water

molecules and bacterial cells. True motility is confirmed by observing the bacterium swim from one side of the microscope field to the other side.

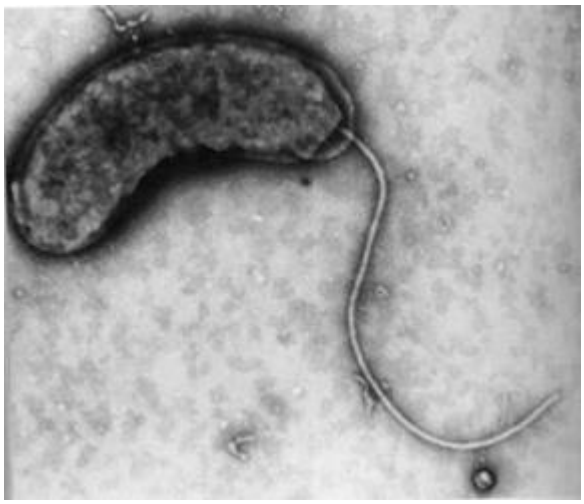


Figure 6. A *Desulfovibrio* species. TEM. About 15,000X. The bacterium is motile by means of a single polar flagellum. Of course, one can determine the presence of flagella by means of electron microscopy. Perhaps this is an alternative way to determine bacterial motility, if you happen to have an electron microscope.

Fimbriae

Fimbriae and **pili** are interchangeable terms used to designate short, hair-like structures on the surfaces of procaryotic cells. Like flagella, they are composed of protein. Fimbriae are shorter and stiffer than flagella, and slightly smaller in diameter. Generally, fimbriae have nothing to do with bacterial movement (there are exceptions, e.g. twitching movement on *Pseudomonas*). Fimbriae are very common in Gram-negative bacteria, but occur in some archaea and Gram-positive bacteria as well. Fimbriae are most often involved in adherence of bacteria to surfaces, substrates and other cells or tissues in nature. In *E. coli*, a specialized type of pilus, the **F or sex pilus**, mediates the transfer of DNA between mating bacteria during the process of **conjugation**, but the function of the smaller, more numerous common pili is quite different.

Common pili (almost always called **fimbriae**) are usually involved in specific adherence (attachment) of procaryotes to surfaces in nature. In medical situations, they are major determinants of bacterial virulence because they allow pathogens to attach to (colonize) tissues and/or to resist attack by phagocytic white blood cells.

For example, pathogenic *Neisseria gonorrhoeae* adheres specifically to the human cervical or urethral epithelium by means of its fimbriae; enterotoxigenic strains of *E. coli* adhere to the mucosal epithelium of the intestine by means of specific fimbriae; the M-protein and associated fimbriae of *Streptococcus pyogenes* are involved in adherence and to resistance to engulfment by phagocytes.

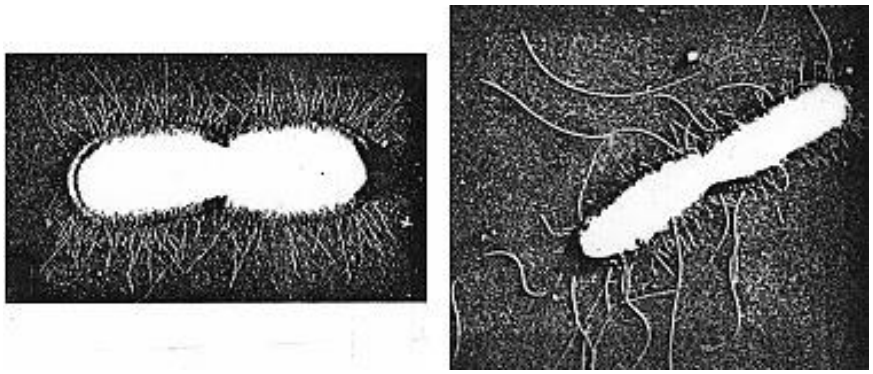


Figure 7. Fimbriae (common pili) and flagella on the surface of bacterial cells. Left: dividing *Shigella* enclosed in fimbriae. The structures are probably involved in the bacterium's ability to adhere to the intestinal surface. Right: dividing pair of *Salmonella* displaying both its peritrichous flagella and its fimbriae. The fimbriae are much shorter and slightly smaller in diameter than flagella. Both *Shigella* and *Salmonella* are enteric bacteria that cause different types of intestinal diarrheas. The bacteria can be differentiated by a motility test. *Salmonella* is motile; *Shigella* is nonmotile.

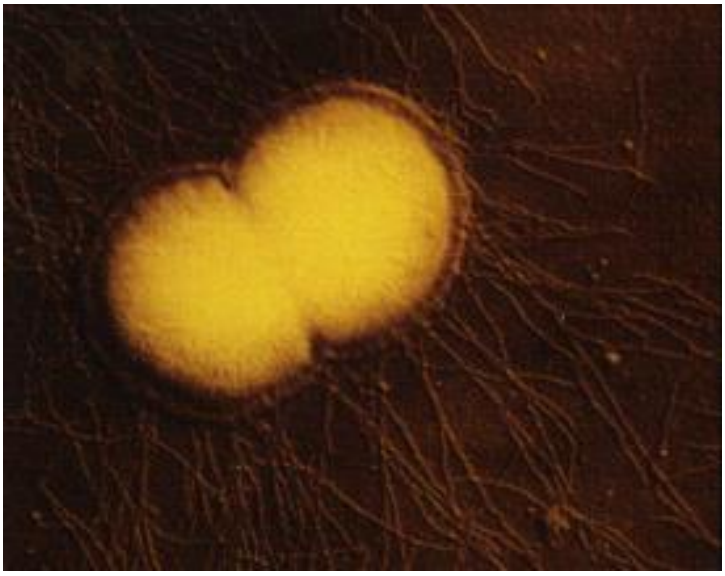


Figure 8. Fimbriae of *Neisseria gonorrhoeae* allow the bacterium to adhere to tissues. Electron micrograph.

Table 2. Some properties of pili and fimbriae.

Bacterial species where observed	Typical number on cell	Distribution on cell surface	Function
Escherichia coli (F or sex pilus)	1-4	uniform	mediates DNA transfer during conjugation
Escherichia coli (common pili or Type 1 fimbriae)	100-200	uniform	surface adherence to epithelial cells of the GI tract
Neisseria gonorrhoeae	100-200	uniform	surface adherence to epithelial cells of the urogenital tract
Streptococcus pyogenes (fimbriae plus the M-protein)	?	uniform	adherence, resistance to phagocytosis; antigenic variability
Pseudomonas aeruginosa	10-20	polar	surface adherence
Sulfolobus acidocaldarius (an archean)	?	?	attachment to sulfur particles

The Cell Envelope

The **cell envelope** is a descriptive term for the several layers of material that envelope or enclose the protoplasm of the cell. The cell protoplasm (**cytoplasm**) is surrounded by the **plasma membrane**, a **cell wall** and a **capsule**. The cell wall itself is a layered structure in Gram-negative bacteria. All cells have a membrane, which is the essential and definitive characteristic of a "cell". Almost all procaryotes have a cell wall to prevent damage to the underlying **protoplast**. Outside the cell wall, foremost as a surface structure, may be a polysaccharide **capsule**, or at least a **glycocalyx**.

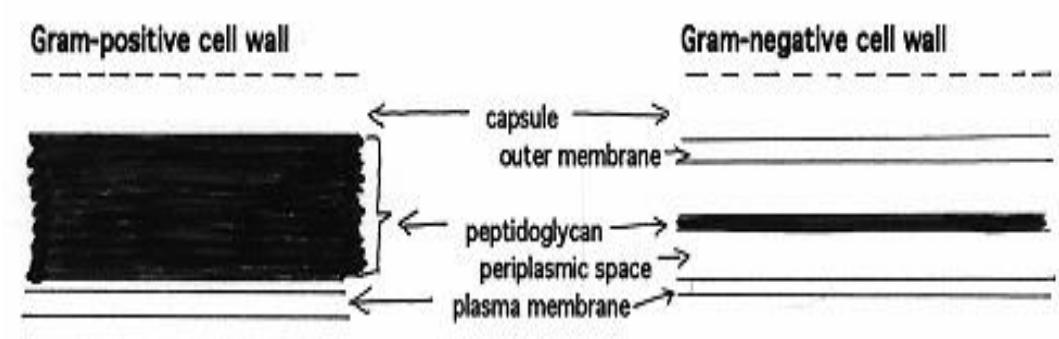


Figure 9. Profiles of the cell envelope the Gram-positive and Gram-negative bacteria. The Gram-positive wall is a uniformly thick layer external to the plasma membrane. It is composed mainly of peptidoglycan (murein). The Gram-negative wall appears thin and multilayered. It consists of a relatively thin peptidoglycan sheet between the plasma membrane and a phospholipid-lipopolysaccharide outer membrane. The space between the inner (plasma) and outer membranes (wherein the peptidoglycan resides) is called the periplasm.

Capsules

Most procaryotes contain some sort of a polysaccharide layer outside of the cell wall polymer. In a general sense, this layer is called a **capsule**. A **true capsule** is a discrete detectable layer of polysaccharides deposited outside the cell wall. A less discrete structure or matrix which embeds the cells is a called a **slime layer** or a **biofilm**. A type of capsule found in bacteria called a **glycocalyx** is a thin layer of tangled polysaccharide fibers which is almost always observed on the surface of cells growing in nature (as opposed to the laboratory).

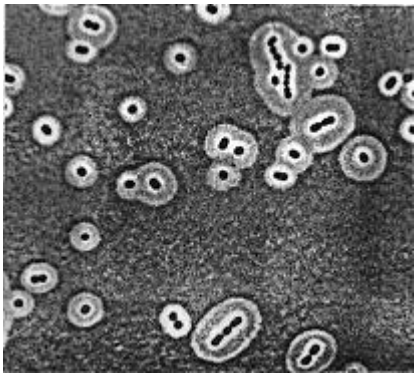


Figure 10. Bacterial capsules outlined by India ink viewed by light microscopy. This is a true capsule, a discrete layer of polysaccharide surrounding the cells. Sometimes bacterial cells are embedded more randomly in a polysaccharide matrix called a slime layer or biofilm. Polysaccharide films that may inevitably be present on the surfaces of bacterial cells, but which cannot be detected visually, are called glycocalyx.

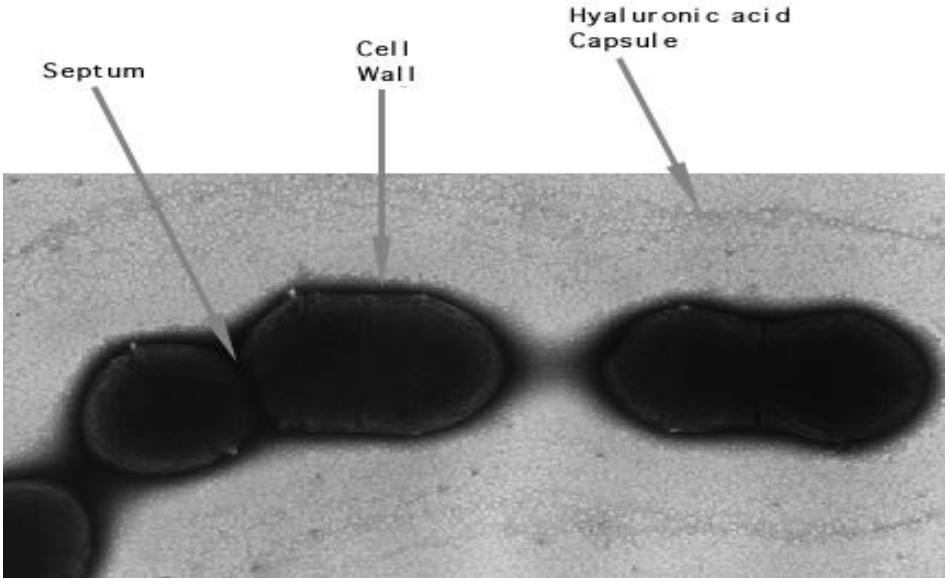


Figure 11. Negative stain of *Streptococcus pyogenes* viewed by transmission electron microscopy (28,000X). The halo around the chain of cells is the hyaluronic acid capsule that surrounds the exterior of the bacteria. The septa between dividing pairs of cells may also be seen. Electron micrograph of *Streptococcus pyogenes*.

Capsules are generally composed of polysaccharide; rarely they contain amino sugars or peptides (see Table 3).

Table 3. Chemical composition of some bacterial capsules.

Bacterium	Capsule composition	Structural subunits
Gram-positive Bacteria		
Bacillus anthracis	polypeptide (polyglutamic acid)	D-glutamic acid
Bacillus megaterium	polypeptide and polysaccharide	D-glutamic acid, amino sugars, sugars
Streptococcus mutans	polysaccharide	(dextran) glucose
Streptococcus pneumoniae	polysaccharides	sugars, amino sugars, uronic acids
Streptococcus pyogenes	polysaccharide (hyaluronic acid)	N-acetyl-glucosamine and glucuronic acid
Gram-negative Bacteria		
Acetobacter xylinum	polysaccharide	(cellulose) glucose
Escherichia coli	polysaccharide (colonic acid)	glucose, galactose, fucose glucuronic acid
Pseudomonas aeruginosa	polysaccharide	mannuronic acid
Azotobacter vinelandii	polysaccharide	glucuronic acid

Agrobacterium tumefaciens	polysaccharide	(glucan) glucose

Capsules have **several functions** and often have multiple functions in a particular organism. Like fimbriae, capsules, slime layers, and glycocalyx often **mediate adherence** of cells to surfaces. Capsules also **protect bacterial cells from engulfment** by predatory protozoa or white blood cells (phagocytes), or from attack by antimicrobial agents of plant or animal origin. Capsules in certain soil bacteria **protect cells from perennial effects of drying** or desiccation. Capsular materials (e.g. dextrans) may be overproduced when bacteria are fed sugars to become **reserves of carbohydrate** for subsequent metabolism.

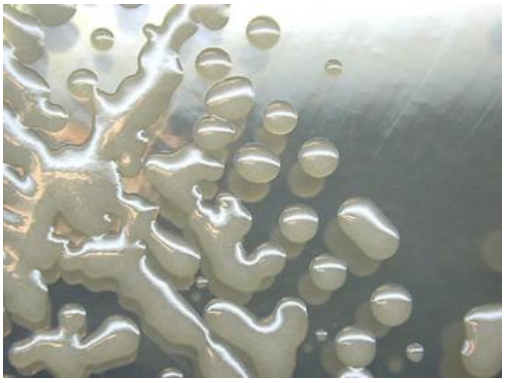


Figure 12. Colonies of *Bacillus anthracis*. The slimy or mucoid appearance of a bacterial colony is usually evidence of capsule production. In the case of *B. anthracis,* the capsule is composed of poly-D-glutamate. The capsule is an essential determinant of virulence to the bacterium. In the early stages of colonization and infection the capsule protects the bacteria from assaults by the immune and phagocytic systems.

Some bacteria produce slime materials to adhere and float themselves as colonial masses in their environments. Other bacteria produce slime materials to attach themselves to a surface or substrate. Bacteria may attach to surface, produce slime, divide and produce microcolonies within the slime layer, and construct a **biofilm**, which becomes an enriched and protected environment for themselves and other bacteria.

A classic example of biofilm construction in nature is the formation of **dental plaque** mediated by the oral bacterium, *Streptococcus mutans*. The bacteria adhere specifically to the pellicle of the tooth by means of a protein on the cell surface. The

bacteria grow and synthesize a dextran capsule which binds them to the enamel and forms a biofilm some 300-500 cells in thickness. The bacteria are able to cleave sucrose (provided by the animal diet) into glucose plus fructose. The fructose is fermented as an energy source for bacterial growth. The glucose is polymerized into an extracellular dextran polymer that cements the bacteria to tooth enamel and becomes the matrix of dental plaque. The dextran slime can be depolymerized to glucose for use as a carbon source, resulting in production of lactic acid within the biofilm (plaque) that decalcifies the enamel and leads to dental caries or bacterial infection of the tooth.

Another important characteristic of capsules may be their ability to block some step in the phagocytic process and thereby prevent bacterial cells from being engulfed or destroyed by phagocytes. For example, the primary determinant of virulence of the pathogen *Streptococcus pneumoniae* is its polysaccharide capsule, which prevents ingestion of pneumococci by alveolar macrophages. *Bacillus anthracis* survives phagocytic engulfment because the lysosomal enzymes of the phagocyte cannot initiate an attack on the poly-D-glutamate capsule of the bacterium. Bacteria such as *Pseudomonas aeruginosa*, that construct a biofilm made of extracellular slime when colonizing tissues, are also resistant to phagocytes, which cannot penetrate the biofilm.

Cell Wall

Most procaryotes have a rigid **cell wall**. The cell wall is an essential structure that protects the cell protoplast from mechanical damage and from osmotic rupture or **lysis**. Procaryotes usually live in relatively dilute environments such that the accumulation of solutes inside the procaryotic cell cytoplasm greatly exceeds the total solute concentration in the outside environment. Thus, the osmotic pressure against the inside of the plasma membrane may be the equivalent of 10-25 atm. Since the membrane is a delicate, plastic structure, it must be restrained by an outside wall made of porous, rigid material that has high tensile strength. Such a material is **murein**, the ubiquitous component of bacterial cell walls.

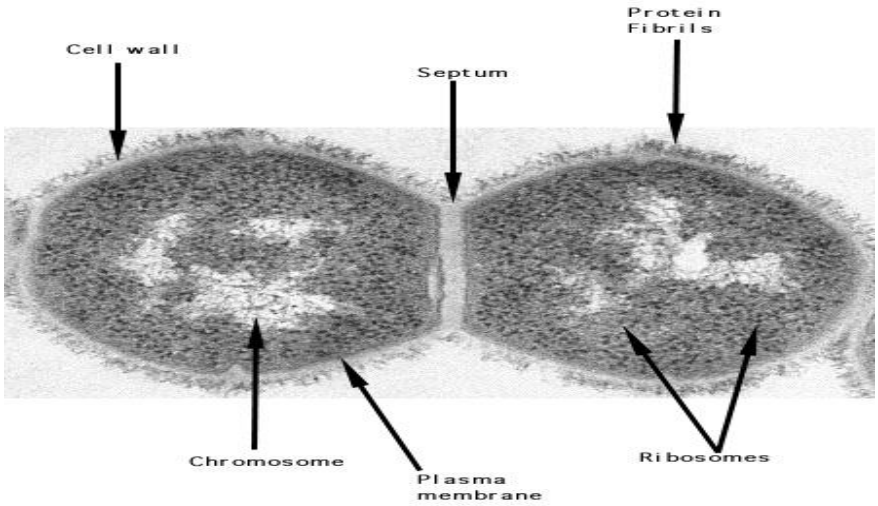


Figure 13. Electron micrograph of an ultra-thin section of a dividing pair of group A streptococci (20,000X). The cell surface fibrils, consisting primarily of M protein, are evident. The bacterial cell wall, to which the fibrils are attached, is also clearly seen as the light staining region between the fibrils and the dark staining cell interior. Cell division in progress is indicated by the new septum formed between the two cells and by the indentation of the cell wall near the cell equator. The streptococcal cell diameter is equal to approximately one micron. Electron micrograph of *Streptococcus pyogenes*.

The cell walls of bacteria deserve special attention for several reasons:

1. They are an essential structure for viability, as described above.
2. They are composed of unique components found nowhere else in nature.
3. They are one of the most important sites for attack by antibiotics.
4. They provide ligands for adherence and receptor sites for drugs or viruses.
5. They cause symptoms of disease in animals.
6. They provide for immunological distinction and immunological variation among strains of bacteria.

The cell walls of all **Bacteria** contain a unique type of **peptidoglycan** called **murein**. Peptidoglycan is a polymer of disaccharides (a glycan) cross-linked by short chains of amino acids (peptides), and many types of peptidoglycan exist. All **Bacterial** peptidoglycans contain **N-acetylmuramic acid**, which is the definitive component of **murein**. The cell walls of **Archaea** may be composed of protein, polysaccharides, or peptidoglycan-like molecules, but never do they contain murein. This feature distinguishes the **Bacteria** from the **Archaea**.

In the **Gram-positive Bacteria** (those that retain the purple crystal violet dye when subjected to the Gram-staining procedure) the cell wall is thick (15-80 nanometers), consisting of several layers of peptidoglycan. In the **Gram-negative Bacteria** (which do not retain the crystal violet) the cell wall is relatively thin (10 nanometers) and is composed of a single layer of peptidoglycan surrounded by a membranous structure called the **outer membrane**. The outer membrane of Gram-negative bacteria invariably contains a unique component, **lipopolysaccharide** (**LPS** or **endotoxin**), which is toxic to animals. In Gram-negative bacteria the outer membrane is usually thought of as part of the cell wall.

Peptidoglycan structure and arrangement in *E. coli* is representative of all *Enterobacteriaceae*, and many other Gram-negative bacteria, as well. The glycan backbone is made up of alternating molecules of N-acetylglucosamine (G) and N-acetylmuramic acid (M) connected by a beta 1,4-glycoside bond. The 3-carbon of N-acetylmuramic acid (M) is substituted with a lactyl ether group derived from pyruvate. The lactyl ether connects the glycan backbone to a peptide side chain that contains L-alanine, (L-ala), D-glutamate (D-glu), Diaminopimelic acid (DAP), and D-alanine (D-ala). MurNAc is unique to bacterial cell walls, as is D-glu, DAP and D-ala. The muramic acid subunit of *E. coli* is shown in Figure 15.

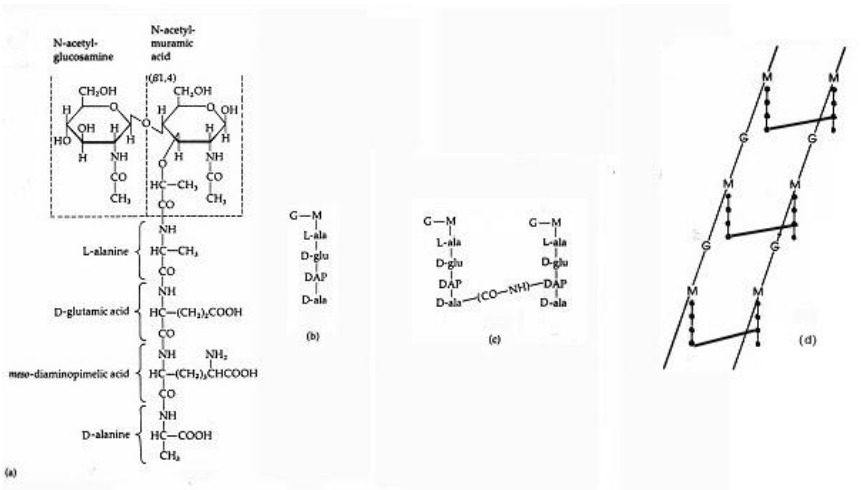


Figure 14. The structure of the muramic acid subunit of the peptidoglycan of *Escherichia coli*. This is the type of murein found in most Gram-negative bacteria. The glycan backbone is a repeat polymer of two amino sugars, N-acetylglucosamine (G) and N-acetylmuramic acid (M). Attached to the N-acetylmuramic acid is a tetrapeptide consisting of L-ala-D-glu-DAP-D-ala.b. Abbreviated structure of the muramic acid subunit. c. Nearby

tetrapeptide side chains may be linked to one another by an interpeptide bond between DAP on one chain and D-ala on the other. d. The polymeric form of the molecule.

Strands of murein are assembled in the periplasm from about 10 muramic acid subunits. Then the strands are connected to form a continuous glycan molecule that encompasses the cell. Wherever their proximity allows it, the tetrapeptide chains that project from the glycan backbone can be cross-linked by an **interpeptide bond** between a free amino group on DAP and a free carboxy group on a nearby D-ala. The assembly of peptidoglycan on the outside of the plasma membrane is mediated by a group of periplasmic enzymes which are transglycosylases, transpeptidases and carboxypeptidases. The mechanism of action of penicillin and related beta-lactam antibiotics is to **block transpeptidase and carboxypeptidase enzymes** during their assembly of the murein cell wall. Hence, the beta lactam antibiotic are said to "block cell wall synthesis" in the bacteria.

The glycan backbone of the peptidoglycan molecule can be cleaved by an enzyme called **lysozyme** that is present in animal serum, tissues and secretions, and in the phagocytic lysosome. The function of lysozyme is to lyse bacterial cells as a constitutive defense against bacterial pathogens. Some Gram-positive bacteria are very sensitive to lysozyme and the enzyme is quite active at low concentrations. Lachrymal secretions (tears) can be diluted 1:40,000 and retain the ability to lyse certain bacterial cells. Gram-negative bacteria are less vulnerable to attack by lysozyme because their peptidoglycan is shielded by the outer membrane. The exact site of lysozymal cleavage is the beta 1,4 bond between N-acetylmuramic acid (M) and N-acetylglucosamine (G) , such that the muramic acid subunit shown in Figure 13(a) is the result of the action of lysozyme on bacterial peptidoglycan.

In Gram-positive bacteria there are numerous different peptide arrangements among peptidoglycans. The best studied is the murein of *Staphylococcus aureus* shown in Figure 16 below. In place of DAP (in *E. coli*) is the diamino acid, L-lysine (L-lys), and in place of the interpeptide bond (in Gram-negatives) is an **interpeptide bridge** of amino acids that connects a free amino group on lysine to a free carboxy group on D-ala of a nearby tetrapeptide side chain. This arrangement apparently allows for more frequent cross-bonding between nearby tetrapeptide side chains. In *S. aureus*,

the interpeptide bridge is a peptide consisting of 5 glycine molecules (called a **pentaglycine bridge**). Assembly of the interpeptide bridge in Gram-positive murein is inhibited by the beta lactam antibiotics in the same manner as the interpeptide bond in Gram-negative murein. Gram-positive bacteria are more sensitive to penicillin than Gram-negative bacteria because the peptidoglycan is not protected by an outer membrane and it is a more abundant molecule. In Gram-positive bacteria, peptidoglycans may vary in the amino acid in place of DAP or L-lys in position 3 of the tetrapeptide, and in the exact composition of the interpeptide bridge. At least eight different types of peptidoglycan exist in Gram-positive bacteria.

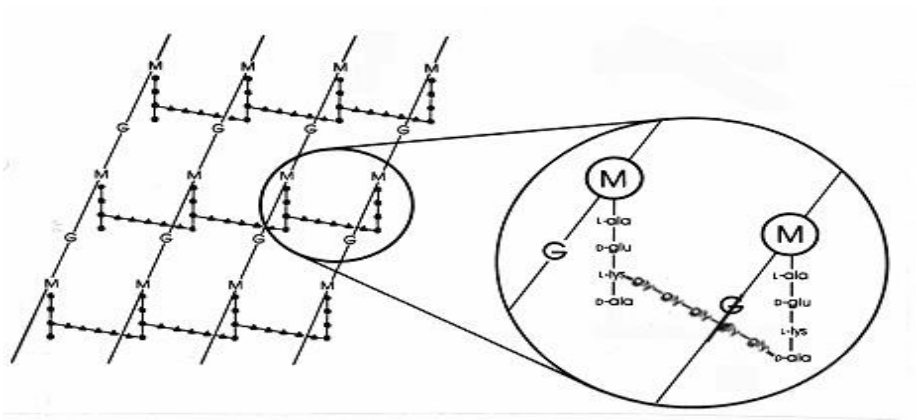


Figure 15. Schematic diagram of the peptidoglycan sheet of *Staphylococcus aureus*. G = N-acetyl-glucosamine; M = N-acetyl-muramic acid; L-ala = L-alanine; D-ala = D-alanine; D-glu = D-glutamic acid; L-lys = L-lysine. This is one type of murein found in Gram-positive bacteria. Compared to the *E. coli* peptidoglycan there is L-lys in place of DAP (diaminopimelic acid) in the tetrapeptide. The free amino group of L-lys is substituted with a glycine pentapeptide (gly-gly-gly-gly-gly-) which then becomes an interpeptide bridge forming a link with a carboxy group from D-ala in an adjacent tetrapeptide side chain. Gram-positive peptidoglycans differ from species to species, mainly in regards to the amino acids in the third position of the tetrapeptide side chain and in the amino acid composition of the interpeptide bridge.

Gram-negative bacteria may contain a single monomolecular layer of murein in their cell walls while Gram-positive bacteria are thought to have several layers or "wraps" of peptidoglycan. Closely associated with the layers of peptidoglycan in Gram-positive bacteria are a group of molecules called teichoic acids. **Teichoic acids** are linear polymers of polyglycerol or polyribitol substituted with phosphates and a few amino acids and sugars. The teichoic acid polymers are occasionally anchored to the plasma membrane (called **lipoteichoic acids**) apparently directed outward at right angles to the layers of peptidoglycan. The functions of teichoic acid are not known.

They are essential to viability of Gram-positive bacteria in the wild. One idea is that they provide a channel of regularly-oriented negative charges for threading positively charged substances through the complicated peptidoglycan network. Another theory is that teichoic acids are in some way involved in the regulation and assembly of muramic acid subunits on the outside of the plasma membrane. There are instances, particularly in the streptococci, wherein teichoic acids have been implicated in the adherence of the bacteria to tissue surfaces.

The Outer Membrane of Gram-negative Bacteria

Of special interest as a component of the Gram-negative cell wall is the **outer membrane**, a discrete bilayered structure on the outside of the peptidoglycan sheet (see Figure 16). For the bacterium, the outer membrane is first and foremost a permeability barrier, but primarily due to its lipopolysaccharide content, it possesses many interesting and important characteristics of Gram-negative bacteria. The outer membrane is a lipid bilayer intercalated with proteins, superficially resembling the plasma membrane. The inner face of the outer membrane is composed of phospholipids similar to the phosphoglycerides that compose the plasma membrane. The outer face of the outer membrane may contain some phospholipid, but mainly it is formed by a different type of amphiphilic molecule which is composed of lipopolysaccharide (LPS). Outer membrane proteins usually traverse the membrane and in one case, anchor the outer membrane to the underlying peptidoglycan sheet.

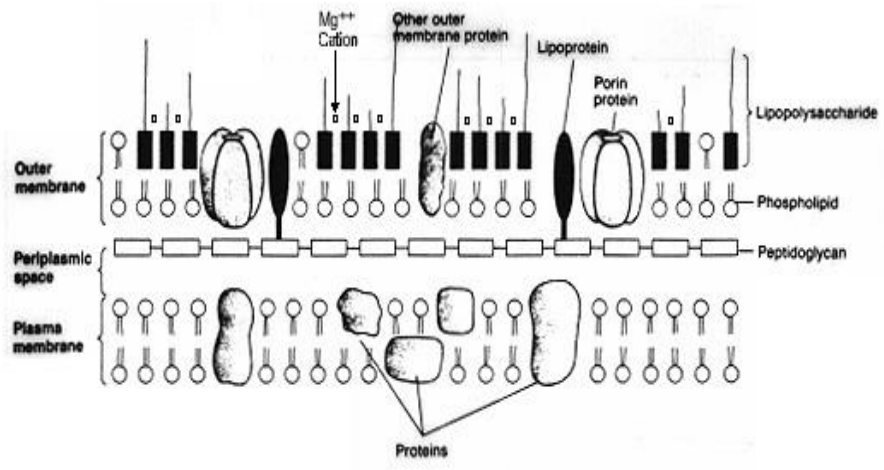


Figure 16. Schematic illustration of the outer membrane, cell wall and plasma membrane of a Gram-negative bacterium. Note the structure and arrangement of molecules that constitute the outer membrane.

The LPS molecule that constitutes the outer face of the outer membrane is composed of a hydrophobic region, called **Lipid A**, that is attached to a hydrophilic linear polysaccharide region, consisting of the **core polysaccharide** and **the O-specific polysaccharide**.

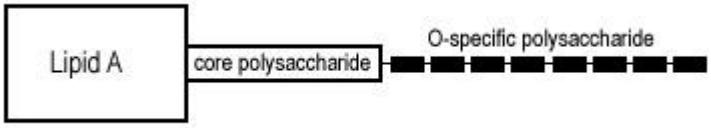


Figure 17. Structure of LPS

The Lipid A head of the molecule inserts into the interior of the membrane, and the polysaccharide tail of the molecule faces the aqueous environment. Where the tail of the molecule inserts into the head there is an accumulation of negative charges such that a magnesium cation is chelated between adjacent LPS molecules. This provides the lateral stability for the outer membrane, and explains why treatment of Gram-negative bacteria with a powerful chelating agent, such as EDTA, causes dispersion of LPS molecules.

Bacterial lipopolysaccharides are toxic to animals. When injected in small amounts LPS or **endotoxin** activates macrophages to produce pyrogens, activates the complement cascade causing inflammation, and activates blood factors resulting in intravascular coagulation and hemorrhage. Endotoxins may play a role in infection by

any Gram-negative bacterium. The toxic component of endotoxin (LPS) is Lipid A. The O-specific polysaccharide may provide ligands for bacterial attachment and confer some resistance to phagocytosis. Variation in the exact sugar content of the O polysaccharide (also referred to as the O antigen) accounts for multiple antigenic types (serotypes) among Gram-negative bacterial pathogens. Therefore. even though Lipid A is the toxic component in LPS, the polysaccharides nonetheless contribute to virulence of Gram-negative bacteria.

The proteins in the outer membrane of *Escherichia coli* are well characterized (see Table 4). About 400,00 copies of the **Braun lipoprotein** are covalently attached to the peptidoglycan sheet at one end and inserted into the hydrophobic interior of the membrane at the opposite end. A group of trimeric proteins called **porins** form pores of a fixed diameter through the lipid bilayer of the membrane. The **omp C** and **omp F** porins of *E. coli* are designed to allow passage of hydrophilic molecules up to mw of about 750 daltons. Larger molecules or harmful hydrophobic compounds (such as bile salts in the intestinal tract) are excluded from entry. Porins are designed in Gram-negative bacteria to allow passage of useful molecules (nutrients) through the barrier of the outer membrane, but to exclude passage harmful substances from the environment. The ubiquitous **omp A** protein in the outer membrane of *E. coli* has a porin like structure, and may function in uptake of specific ions, but it is also a receptor for the F pilus and an attachment site for bacterial viruses.

Table 4. Functions of the outer membrane components of *Escherichia coli*.

Component	Function
Lipopolysaccharide (LPS)	Permeability barrier
Mg++ bridges	Stabilizes LPS and is essential for its permeability characteristics
Braun lipoprotein	Anchors the outer membrane to peptidoglycan (murein) sheet
Omp C and Omp F porins	proteins that form pores or channels through outer membrane for passage of hydrophilic molecules
Omp A protein	provides receptor for some viruses and bacteriocins; stabilizes mating cells

	during conjugation

A correlation between Gram stain reaction and cell wall properties of bacteria is summarized in Table 5. The Gram stain procedure contains a "destaining" step wherein the cells are washed with an acetone-alcohol mixture. The lipid content of the Gram-negative wall probably affects the outcome of this step so that Gram-positive cells retain a primary stain while Gram-negative cells are destained.

Table 5. Correlation of Grams stain with other properties of Bacteria.

Property	Gram-positive	Gram-negative
Thickness of wall	thick (20-80 nm)	thin (10 nm)
Number of layers	1	2
Peptidoglycan (murein) content	>50%	10-20%
Teichoic acids in wall	present	absent
Lipid and lipoprotein content	0-3%	58%
Protein content	0	9%
Lipopolysaccharide content	0	13%
Sensitivity to Penicillin G	yes	no (1)
Sensitivity to lysozyme	yes	no (2)

(1) A few Gram-negative bacteria are sensitive to natural penicillins. Many Gram-negative bacteria are sensitive to some type of penicillin, especially semisynthetic penicillins. Gram-negative bacteria, including *E. coli*, can be made sensitive to natural penicillin by procedures that disrupt the permeability characteristics of the outer membrane.

(2) Gram-negative bacteria are sensitive to lysozyme if pretreated by some procedure that removes the outer membrane and exposes the peptidoglycan directly to the enzyme.

Cell Wall-less Forms

A few bacteria are able to live or exist without a cell wall. The mycoplasmas are a group of bacteria that lack a cell wall. Mycoplasmas have sterol-like molecules

incorporated into their membranes and they are usually inhabitants of osmotically-protected environments. *Mycoplasma pneumoniae* is the cause of primary atypical bacterial pneumonia, known in the vernacular as "walking pneumonia". For obvious reasons, penicillin is ineffective in treatment of this type of pneumonia. Sometimes, under the pressure of antibiotic therapy, pathogenic streptococci can revert to cell wall-less forms (called **spheroplasts**) and persist or survive in osmotically-protected tissues. When the antibiotic is withdrawn from therapy the organisms may regrow their cell walls and reinfect unprotected tissues.

The Plasma Membrane

The **plasma membrane**, also called the **cytoplasmic membrane**, is the most dynamic structure of a procaryotic cell. Its main function is a s a **selective permeability barrier** that regulates the passage of substances into and out of the cell. The plasma membrane is the definitive structure of a cell since it sequesters the molecules of life in a unit, separating it from the environment. The bacterial membrane allows passage of water and uncharged molecules up to mw of about 100 daltons, but does not allow passage of larger molecules or any charged substances except by means special membrane **transport processes** and **transport systems**.

Since procaryotes lack any intracellular organelles for processes such as respiration or photosynthesis or secretion, the plasma membrane subsumes these processes for the cell and consequently has a variety of functions in **energy generation**, and **biosynthesis**. For example, the **electron transport system** that couples **aerobic respiration** and **ATP synthesis** is found in the procaryotic membrane. The **photosynthetic chromophores** that harvest light energy for conversion into chemical energy are located in the membrane. Hence, the plasma membrane is the site of **oxidative phosphorylation** and **photophosphorylation** in procaryotes, analogous to the functions of mitochondria and chloroplasts in eukaryotic cells. Besides **transport proteins** that selectively mediate the passage of substances into and out of the cell, procaryotic membranes may contain **sensing proteins** that measure concentrations of molecules in the environment or **binding proteins** that translocate signals to genetic and metabolic machinery in the cytoplasm. Membranes

also contain **enzymes** involved in many metabolic processes such as cell wall synthesis, septum formation, membrane synthesis, DNA replication, CO_2 fixation and ammonia oxidation. The predominant functions of bacterial membranes are listed in the table below.

Table 6. Functions of the procaryotic plasma membrane.

1. Osmotic or permeability barrier

2. Location of transport systems for specific solutes (nutrients and ions)

3. Energy generating functions, involving respiratory and photosynthetic electron transport systems, establishment of proton motive force, and transmembranous, ATP-synthesizing ATPase

4. Synthesis of membrane lipids (including lipopolysaccharide in Gram-negative cells)

5. Synthesis of murein (cell wall peptidoglycan)

6. Assembly and secretion of extracytoplasmic proteins

7. Coordination of DNA replication and segregation with septum formation and cell division

8. Chemotaxis (both motility per se and sensing functions)

9. Location of specialized enzyme system

Bacterial membranes are composed of 40 percent phospholipid and 60 percent protein. The phospholipids are amphoteric molecules with a polar hydrophilic glycerol "head" attached via an ester bond to two nonpolar hydrophobic fatty acid tails, which naturally form a bilayer in aqueous environments. Dispersed within the bilayer are various structural and enzymatic proteins which carry out most membrane functions. At one time, it was thought that the proteins were neatly organized along the inner and outer faces of the membrane and that this accounted for the double track

appearance of the membrane in electron micrographs. However, it is now known that while some membrane proteins are located and function on one side or another of the membrane, most proteins are partly inserted into the membrane, or possibly even traverse the membrane as channels from the outside to the inside. It is possible that proteins can move laterally along a surface of the membrane, but it is thermodynamically unlikely that proteins can be rotated within a membrane, which discounts early theories of how transport systems might work. The arrangement of proteins and lipids to form a membrane is called the **fluid mosaic model**, and is illustrated in Figure 18.

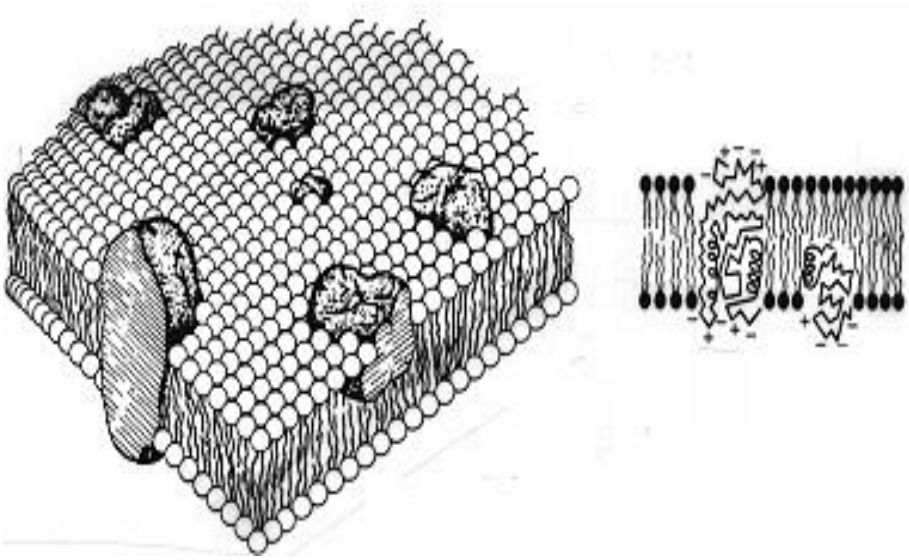


Figure 18. Fluid mosaic model of a biological membrane. In aqueous environments membrane phospholipids arrange themselves in such a way that they spontaneously form a fluid bilayer. Membrane proteins, which may be structural or functional, may be permanently or transiently associated with one side or the other of the membrane, or even be permanently built into the bilayer, while other proteins span the bilayer and may form transport channels through the membrane.

Transport Processes

The proteins that mediate the passage of solutes through membranes are referred to variously as **transport systems**, **carrier proteins**, **porters**, and **permeases**. Transport systems operate by one of three **transport processes** as described below in Figure 19. In a **uniport** process, a solute passes through the membrane

unidirectionally. In **symport** processes (also called **cotransport**) two solutes must be transported in the same direction at the same time; in **antiport** processes (also called **exchange diffusion**), one solute is transported in one direction simultaneously as a second solute is transported in the opposite direction.

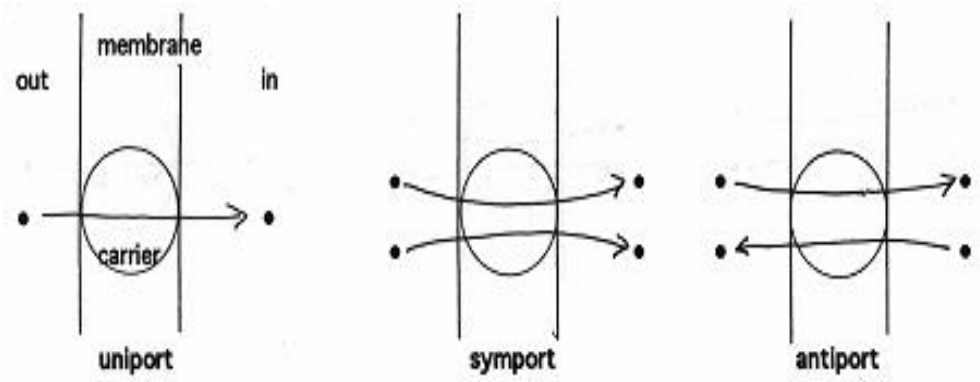


Figure 19. Transport processes in bacterial cells. Solutes enter or exit from bacterial cells by means of one of three processes: uniport, symport (also called cotransport) and antiport (also called exchange diffusion). Transport systems (Figure 20 below) operate by one or another of these processes.

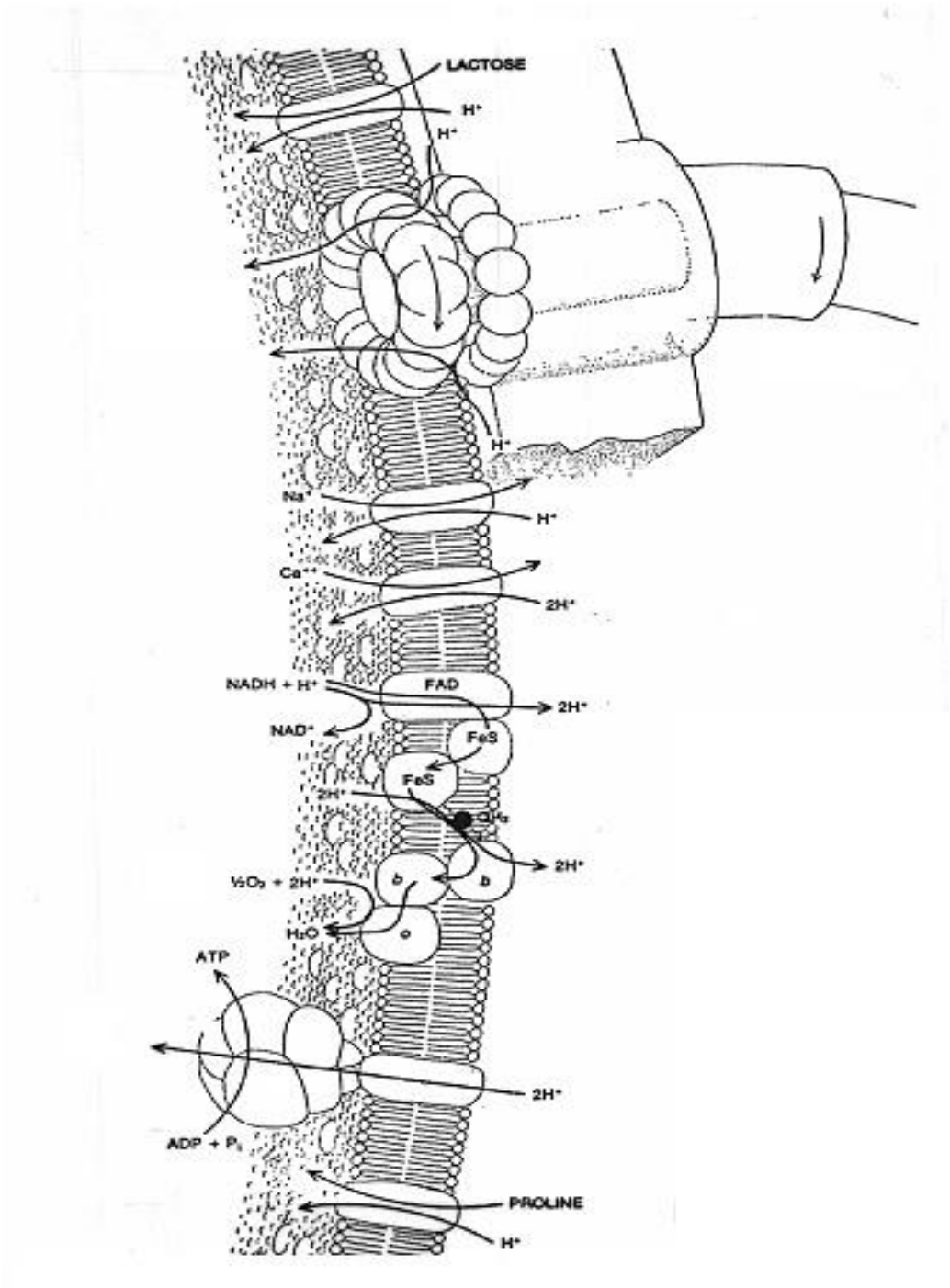


Figure 20. Schematic view of the plasma membrane of *Escherichia coli*. The S and M rings which constitute the flagellar motor are shown. The motor ring is imbedded in the phospholipid bilayer. It is powered by pmf to rotate the flagellar filament. The electron transport system is shown oxidizing NAD by removal of a pair of electrons, passing them through its sequence of carriers eventually to O2. ATPase is the transmembranous protein enzyme that utilizes protons from the outside to synthesize ATP on the inside of the membrane. Several other transmembranous proteins are

transport systems which are operating by either symport or antiport processes.

The Cytoplasm

The cytoplasmic constituents of procaryotic cells invariably include the **procaryotic chromosome** and **ribosomes**. The chromosome is typically one large circular molecule of **DNA**, more or less free in the cytoplasm. Procaryotes sometimes possess smaller extrachromosomal pieces of DNA called **plasmids**. The total DNA content of a procaryote is referred to as the cell **genome**. During cell growth and division, the procaryotic chromosome is replicated in the usual semi-conservative fashion before for distribution to progeny cells. However, the eukaryotic processes of meiosis and mitosis are absent in procaryotes. Replication and segregation of procaryotic DNA is coordinated by the membrane, possibly by mesosomes.

The distinct granular appearance of procaryotic cytoplasm is due to the presence and distribution of **ribosomes**. The ribosomes of procaryotes are smaller than cytoplasmic ribosomes of eukaryotes. procaryotic ribosomes are 70S in size, being composed of 30S and 50S subunits. The 80S ribosomes of eukaryotes are made up of 40S and 60S subunits. Ribosomes are involved in the process of translation (protein synthesis), but some details of their activities differ in eukaryotes, Bacteria and Archaea. Protein synthesis using 70S ribosomes occurs in eukaryotic mitochondria and chloroplasts, and this is taken as a major line of evidence that these organelles are descended from procaryotes.

Table 9. Inorganic ions present in a growing bacterial cell.

Ion	Function
K^+	Maintenance of ionic strength; cofactor for certain enzymes
NH_4^+	Principal form of inorganic N for assimilation
Ca^{++}	Cofactor for certain enzymes
Fe^{++}	Present in cytochromes and other metalloenzymes
Mg^{++}	Cofactor for many enzymes; stabilization of outer membrane of Gram-negative bacteria
Mn^{++}	Present in certain metalloenzymes

Co^{++}	Trace element constituent of vitamin B12 and its coenzyme derivatives and found in certain metalloenzymes
Cu^{++}	Trace element present in certain metalloenzymes
Mo^{++}	Trace element present in certain metalloenzymes
Ni^{++}	Trace element present in certain metalloenzymes
Zn^{++}	Trace element present in certain metalloenzymes
SO_4^{--}	Principal form of inorganic S for assimilation
PO_4^{---}	Principal form of P for assimilation and a participant in many metabolic reactions

Inclusions

Often contained in the cytoplasm of procaryotic cells is one or another of some type of inclusion granule. **Inclusions** are distinct granules that may occupy a substantial part of the cytoplasm. Inclusion granules are usually reserve materials of some sort. For example, carbon and energy reserves may be stored as glycogen (a polymer of glucose) or as polybetahydroxybutyric acid (a type of fat) granules. Polyphosphate inclusions are reserves of PO_4 and possibly energy; elemental sulfur (sulfur globules) are stored by some phototrophic and some lithotrophic procaryotes as reserves of energy or electrons. Some inclusion bodies are actually membranous vesicles or intrusions into the cytoplasm which contain photosynthetic pigments or enzymes.

Table 10. Some inclusions in bacterial cells.

Cytoplasmic inclusions	Where found	Composition	Function
glycogen	many bacteria e.g. *E. coli*	polyglucose	reserve carbon and energy source
polybetahydroxyutyric acid (PHB)	many bacteria e.g. *Pseudomonas*	polymerized hydroxy butyrate	reserve carbon and energy source
polyphosphate (volutin granules)	many bacteria e.g. *Corynebacterium*	linear or cyclical polymers of PO4	reserve phosphate; possibly a reserve of high energy phosphate
sulfur globules	phototrophic purple and	elemental sulfur	reserve of electrons

	green sulfur bacteria and lithotrophic colorless sulfur bacteria		(reducing source) in phototrophs; reserve energy source in lithotrophs
gas vesicles	aquatic bacteria especially cyanobacteria	protein hulls or shells inflated with gases	buoyancy (floatation) in the vertical water column
parasporal crystals	endospore-forming bacilli (genus *Bacillus*)	protein	unknown but toxic to certain insects
magnetosomes	certain aquatic bacteria	magnetite (iron oxide) Fe3O4	orienting and migrating along geo- magnetic field lines
carboxysomes	many autotrophic bacteria	enzymes for autotrophic CO2 fixation	site of CO2 fixation
phycobilisomes	cyanobacteria	phycobiliproteins	light-harvesting pigments
chlorosomes	Green bacteria	lipid and protein and bacteriochlorophyll	light-harvesting pigments and antennae

Endospores

A bacterial structure sometimes observed as an inclusion is actually a type of dormant cell called an **endospore**. Endospores are formed by a few groups of **Bacteria** as intracellular structures, but ultimately they are released as free endospores. Biologically, endospores are a fascinating type of cell. Endospores exhibit no signs of life, being described as **cryptobiotic**. They are highly resistant to environmental stresses such as high temperature (some endospores can be boiled for hours and retain their viability), irradiation, strong acids, disinfectants, etc. They are probably the most durable cell produced in nature. Although cryptobiotic, they retain viability indefinitely such that under appropriate environmental conditions, they germinate back into vegetative cells. Endospores are formed by vegetative cells in response to environmental signals that indicate a limiting factor for vegetative growth, such as exhaustion of an essential nutrient. They germinate and become vegetative cells when the environmental stress is relieved. Hence, endospore-formation is a mechanism of survival rather than a mechanism of reproduction.

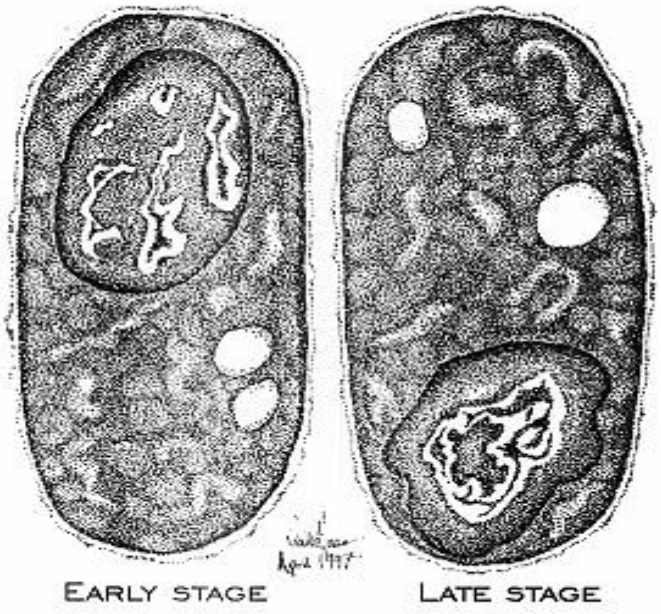


Figure 21. Early and late stages of endospore formation. Drawing by Vaike Haas, University of Wisconsin Madison. During endospore formation, a vegetative cell is converted to a heat-resistant spore. There are eight stages, O,I-VII, in the sporulation cycle of a *Bacillus* species, and the process takes about eight hours. During the early stages (Stage II,) one bacterial chromosome and a few ribosomes are partitioned off by the bacterial membrane to form a protoplast within the mother cell. By the late stages (Stage VI) the protoplast (now called a forespore) has developed a second membrane and several wall-like layers of material are deposited between the two membranes.

Table 11. Differences between endospores and vegetative cells.

Property	Vegetative cells	Endospores
Surface coats	Typical Gram-positive murein cell wall polymer	Thick spore coat, cortex, and peptidoglycan core wall
Microscopic appearance	Nonrefractile	Refractile
Calcium dipicolinic acid	Absent	Present in core
Cytoplasmic water activity	High	Very low
Enzymatic activity	Present	Absent
Macromolecular synthesis	Present	Absent

Heat resistance	Low	High
Resistance to chemicals and acids	Low	High
Radiation resistance	Low	High
Sensitivity to lysozyme	Sensitive	Resistant
Sensitivity to dyes and staining	Sensitive	Resistant

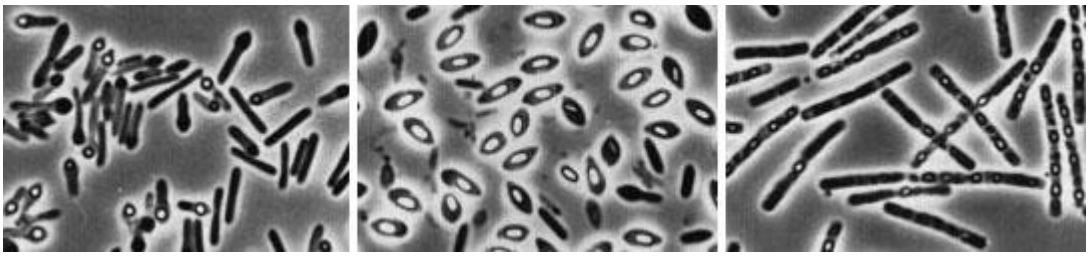


Figure 22. Bacterial endospores. Phase microscopy of sporulating bacteria demonstrates the refractility of endospores, as well as characteristic spore shapes and locations within the mother cell.

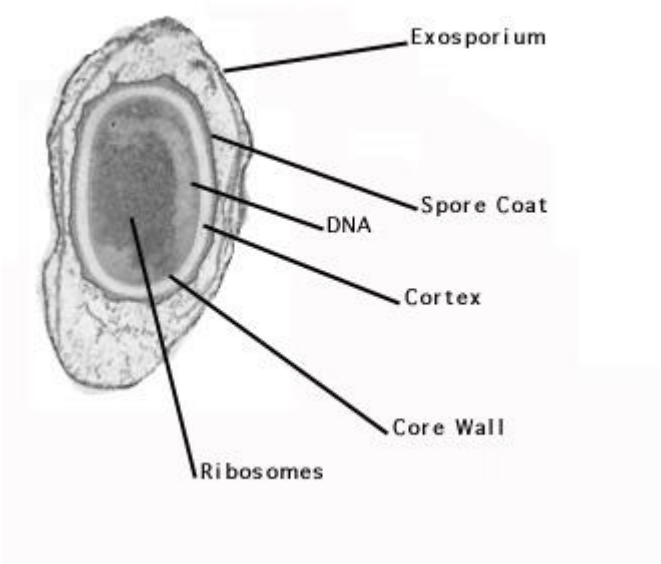


Figure 23. Electron micrograph of a bacterial endospore. The spore has a core wall of unique peptidoglycan surrounded by several layers, including the cortex, the spore coat and the exosporium. The dehydrated core contains the bacterial chromosome and a few ribosomes and enzymes to jump-start protein synthesis and metabolism during germination.

NUTRITION AND GROWTH OF BACTERIA

Nutritional Requirements of Cells

Every organism must find in its environment all of the substances required for energy generation and cellular biosynthesis. The chemicals and elements of this environment that are utilized for bacterial growth are referred to as **nutrients** or **nutritional requirements**. In the laboratory, bacteria are grown in **culture media** which are designed to provide all the essential nutrients in solution for bacterial growth.

The Major Elements

At an elementary level, the nutritional requirements of a bacterium such as *E. coli* are revealed by the cell's elemental composition, which consists of C, H, O, N, S. P, K, Mg, Fe, Ca, Mn, and traces of Zn, Co, Cu, and Mo. These elements are found in the form of water, inorganic ions, small molecules, and macromolecules which serve either a structural or functional role in the cells. The general physiological functions of the elements are outlined in Table 1 below.

Table 1. Major elements, their sources and functions in bacterial cells.

Element	% of dry weight	Source	Function
Carbon	50	organic compounds or CO_2	Main constituent of cellular material
Oxygen	20	H_2O, organic compounds, CO_2, and O_2	Constituent of cell material and cell water; O_2 is electron acceptor in aerobic respiration
Nitrogen	14	NH_3, NO_3, organic compounds, N_2	Constituent of amino acids, nucleic acids nucleotides, and coenzymes
Hydrogen	8	H_2O, organic compounds, H_2	Main constituent of organic compounds and cell water
Phosphorus	3	inorganic phosphates	Constituent of nucleic acids, nucleotides,

		(PO_4)	phospholipids, LPS, teichoic acids
Sulfur	1	SO_4, H_2S, S^o, organic sulfur compounds	Constituent of cysteine, methionine, glutathione, several coenzymes
Potassium	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Iron	0.2	Iron salts	Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions

Trace Elements

Table 1 ignores the occurrence of trace elements in bacterial nutrition. **Trace elements** are metal ions required by certain cells in such small amounts that it is difficult to detect (measure) them, and it is not necessary to add them to culture media as nutrients. Trace elements are required in such small amounts that they are present as "contaminants" of the water or other media components. As metal ions, the trace elements usually act as cofactors for essential enzymatic reactions in the cell. One organism's trace element may be another's required element and vice-versa, but the usual cations that qualify as trace elements in bacterial nutrition are Mn, Co, Zn, Cu, and Mo.

Carbon and Energy Sources for Bacterial Growth

In order to grow in nature or in the laboratory, a bacterium must have an energy source, a source of carbon and other required nutrients, and a permissive range of physical conditions such as O_2 concentration, temperature, and pH. Sometimes bacteria are referred to as individuals or groups based on their patterns of growth under various chemical (nutritional) or physical conditions. For example, phototrophs

are organisms that use light as an energy source; anaerobes are organisms that grow without oxygen; thermophiles are organisms that grow at high temperatures.

All living organisms require a source of energy. Organisms that use radiant energy (light) are called **phototrophs**. Organisms that use (oxidize) an organic form of carbon are called **heterotrophs** or **chemo(hetero)trophs**. Organisms that oxidize inorganic compounds are called **lithotrophs**.

The carbon requirements of organisms must be met by organic carbon (a chemical compound with a carbon-hydrogen bond) or by CO_2. Organisms that use organic carbon are **heterotrophs** and organisms that use CO_2 as a sole source of carbon for growth are called **autotrophs**.

Thus, on the basis of carbon and energy sources for growth four major nutritional types of procaryotes may be defined (Table 2).

Table 2. Major nutritional types of procaryotes

Nutritional Type	Energy Source	Carbon Source	Examples
Photoautotrophs	Light	CO_2	Cyanobacteria, some Purple and Green Bacteria
Photoheterotrophs	Light	Organic compounds	Some Purple and Green Bacteria
Chemoautotrophs or Lithotrophs (Lithoautotrophs)	Inorganic compounds, e.g. H_2, NH_3, NO_2, H_2S	CO_2	A few Bacteria and many Archaea
Chemoheterotrophs or Heterotrophs	Organic compounds	Organic compounds	Most Bacteria, some Archaea

Almost all eukaryotes are either photoautotrophic (e.g. plants and algae) or heterotrophic (e.g. animals, protozoa, fungi). Lithotrophy is unique to procaryotes and photoheterotrophy, common in the Purple and Green Bacteria, occurs only in a very few eukaryotic algae. Phototrophy has not been found in the Archaea, except for nonphotosynthetic light-driven ATP synthesis in the extreme halophiles.

Growth Factors

This simplified scheme for use of carbon, either organic carbon or CO_2, ignores the possibility that an organism, whether it is an autotroph or a heterotroph, may require small amounts of certain organic compounds for growth because they are essential substances that the organism is unable to synthesize from available nutrients. Such compounds are called **growth factors**.

Growth factors are required in small amounts by cells because they fulfill specific roles in biosynthesis. The need for a growth factor results from either a blocked or missing metabolic pathway in the cells. Growth factors are organized into three categories.

1. **purines and pyrimidines**: required for synthesis of nucleic acids (DNA and RNA)

2. **amino acids**: required for the synthesis of proteins

3. **vitamins**: needed as coenzymes and functional groups of certain enzymes

Some bacteria (e.g *E. coli*) do not require any growth factors: they can synthesize all essential purines, pyrimidines, amino acids and vitamins, starting with their carbon source, as part of their own intermediary metabolism. Certain other bacteria (e.g. *Lactobacillus*) require purines, pyrimidines, vitamins and several amino acids in order to grow. These compounds must be added in advance to culture media that are used to grow these bacteria. The growth factors are not metabolized directly as sources of carbon or energy, rather they are assimilated by cells to fulfill their specific role in metabolism. Mutant strains of bacteria that require some growth factor not needed by the wild type (parent) strain are referred to as **auxotrophs**. Thus, a strain of *E. coli* that requires the amino acid tryptophan in order to grow would be called a tryptophan auxotroph and would be designated *E. coli* ***trp-.***

Some vitamins that are frequently required by certain bacteria as growth factors are listed in Table 3. The function(s) of these vitamins in essential enzymatic reactions

gives a clue why, if the cell cannot make the vitamin, it must be provided exogenously in order for growth to occur.

Table 3. Common vitamins required in the nutrition of certain bacteria.

Vitamin	Coenzyme form	Function
p-Aminobenzoic acid (PABA)	-	Precursor for the biosynthesis of folic acid
Folic acid	Tetrahydrofolate	Transfer of one-carbon units and required for synthesis of thymine, purine bases, serine, methionine and pantothenate
Biotin	Biotin	Biosynthetic reactions that require CO_2 fixation
Lipoic acid	Lipoamide	Transfer of acyl groups in oxidation of keto acids
Mercaptoethane-sulfonic acid	Coenzyme M	CH_4 production by methanogens
Nicotinic acid	NAD (nicotinamide adenine dinucleotide) and NADP	Electron carrier in dehydrogenation reactions
Pantothenic acid	Coenzyme A and the Acyl Carrier Protein (ACP)	Oxidation of keto acids and acyl group carriers in metabolism
Pyridoxine (B_6)	Pyridoxal phosphate	Transamination, deamination, decarboxylation and racemation of amino acids
Riboflavin (B_2)	FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide)	Oxidoreduction reactions
Thiamine (B_1)	Thiamine pyrophosphate (TPP)	Decarboxylation of keto acids and transaminase reactions
Vitamin B_{12}	Cobalamine coupled to adenine nucleoside	Transfer of methyl groups
Vitamin K	Quinones and napthoquinones	Electron transport processes

Culture Media for the Growth of Bacteria

For any bacterium to be propagated for any purpose it is necessary to provide the appropriate biochemical and biophysical environment. The biochemical (nutritional)

environment is made available as a **culture medium**, and depending upon the special needs of particular bacteria (as well as particular investigators) a large variety and types of culture media have been developed with different purposes and uses. Culture media are employed in the isolation and maintenance of pure cultures of bacteria and are also used for identification of bacteria according to their biochemical and physiological properties.

The manner in which bacteria are cultivated, and the purpose of culture media, varies widely. **Liquid media** are used for growth of pure batch cultures, while solidified media are used widely for the isolation of pure cultures, for estimating viable bacterial populations, and a variety of other purposes. The usual gelling agent for solid or **semisolid medium** is **agar**, a hydrocolloid derived from red algae. Agar is used because of its unique physical properties (it melts at 100 degrees and remains liquid until cooled to 40 degrees, the temperature at which it gels) and because it cannot be metabolized by most bacteria. Hence as a medium component it is relatively inert; it simply holds (gels) nutrients that are in aquaeous solution.

Types of Culture Media

Culture media may be classified into several categories depending on their composition or use. A **chemically-defined (synthetic) medium** (Table 4a and 4b) is one in which the exact chemical composition is known. A **complex (undefined) medium** (Table 5a and 5b) is one in which the exact chemical constitution of the medium is not known. Defined media are usually composed of pure biochemicals off the shelf; complex media usually contain complex materials of biological origin such as blood or milk or yeast extract or beef extract, the exact chemical composition of which is obviously undetermined. A defined medium is a **minimal medium** (Table4a) if it provides only the exact nutrients (including any growth factors) needed by the organism for growth. The use of defined minimal media requires the investigator to know the exact nutritional requirements of the organisms in question. Chemically-defined media are of value in studying the minimal

nutritional requirements of microorganisms, for enrichment cultures, and for a wide variety of physiological studies. Complex media usually provide the full range of growth factors that may be required by an organism so they may be more handily used to cultivate unknown bacteria or bacteria whose nutritional requirement are complex (i.e., organisms that require a lot of growth factors, known or unknown).

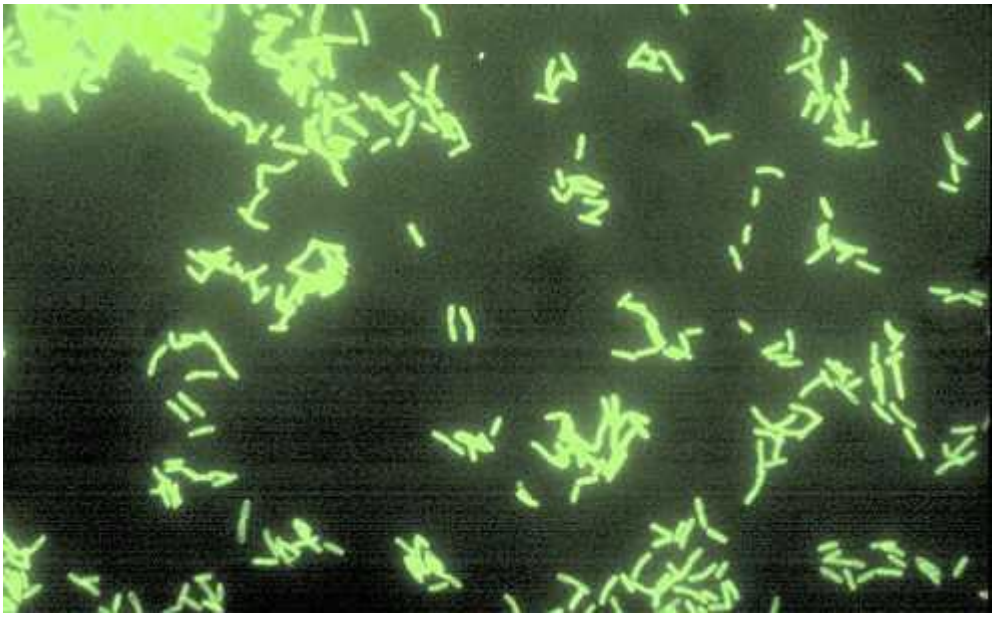


Figure 1. *Legionella pneumophila.* Direct fluorescent antibody (DFA) stain of a patient respiratory tract specimen. In spite of its natural ocurrence in water cooling towers and air conditioners, *Legionella* is a fastidious bacterium grown in the laboratory, which led to the long lag in identification of the first outbreak of Legionaire's disease in Philadelphia in 1977. Had fluoresent antibody to the bacterium been available at that time, diagnosis could have been made as quickly as the time to prepare and view this slide.

Most pathogenic bacteria of animals, which have adapted themselves to growth in animal tissues, require complex media for their growth. Blood, serum and tissue extracts are frequently added to culture media for the cultivation of pathogens. Even so, for a few fastidious pathogens such as *Treponema pallidum*, the agent of syphilis, and *Mycobacterium leprae*, the cause of leprosy, artificial culture media and conditions have not been established. This fact thwarts the the ability to do basic research on these pathogens and the diseases that they cause.

Other concepts employed in the construction of culture media are the principles of selection and enrichment. A **selective medium** is one which has a component(s) added to it which will inhibit or prevent the growth of certain types or species of bacteria and/or promote the growth of desired species. One can also adjust the physical conditions of a culture medium, such as pH and temperature, to render it selective for organisms that are able to grow under these certain conditions.

A culture medium may also be a **differential medium** if allows the investigator to distinguish between different types of bacteria based on some observable trait in their pattern of growth on the medium. Thus a **selective, differential medium** for the isolation of *Staphylococcus aureus,* the most common bacterial pathogen of humans, contains a very high concentration of salt (which the staph will tolerate) that inhibits most other bacteria, mannitol as a source of fermentable sugar, and a pH indicator dye. From clinical specimens, only staph will grow. *S. aureus* is differentiated from *S. epidermidis* (a nonpathogenic component of the normal flora) on the basis of its ability to ferment mannitol. Mannitol-fermenting colonies *(S. aureus*)produce acid which reacts with the indicator dye forming a colored halo around the colonies; mannitol non-fermenters (*S. epidermidis*) use other non-fermentative substrates in the medium for growth and do not form a halo around their colonies.

An enrichment medium employs a slightly different twist. An **enrichment medium** (Table 5a and 5b) contains some component that permits the growth of specific types or species of bacteria, usually because they alone can utilize the component from their environment. However, an enrichment medium may have selective features. An enrichment medium for nonsymbiotic nitrogen-fixing bacteria omits a source of added nitrogen to the medium. The medium is inoculated with a potential source of these bacteria (e.g. a soil sample) and incubated in the atmosphere wherein the only source of nitrogen available is N_2. A selective enrichment medium (Table 5b) for growth of the extreme halophile (*Halococcus*) contains nearly 25 percent salt [NaCl], which is required by the extreme halophile and which inhibits the growth of all other procaryotes.

Table 4a. Minimal medium for the growth of *Bacillus megaterium.* An example of a chemically-defined medium for growth of a heterotrophic bacterium.

Component	Amount	Function of component
sucrose	10.0 g	C and energy source
K_2HPO_4	2.5 g	pH buffer; P and K source
KH_2PO_4	2.5 g	pH buffer; P and K source
$(NH_4)2HPO_4$	1.0 g	pH buffer; N and P source
$MgSO_4$ $7H_2O$	0.20 g	S and Mg^{++} source
$FeSO_4$ $7H_2O$	0.01 g	Fe^{++} source
$MnSO_4$ $7H_2O$	0.007 g	Mn^{++} Source
water	985 ml	
pH 7.0		

Table 4b. Defined medium (also an enrichment medium) for the growth of *Thiobacillus thiooxidans,* a lithoautotrophic bacterium.

Component	Amount	Function of component
NH_4Cl	0.52 g	N source
KH_2PO_4	0.28 g	P and K source
$MgSO_4$ $7H_2O$	0.25 g	S and Mg^{++} source
$CaCl_2$ $2H_2O$	0.07 g	Ca^{++} source
Elemental Sulfur	1.56 g	Energy source
CO_2	5%*	C source
water	1000 ml	
pH 3.0		

* Aerate medium intermittently with air containing 5% CO_2.

Table 5a. Complex medium for the growth of fastidious bacteria.

Component	Amount	Function of component
Beef extract	1.5 g	Source of vitamins and other growth factors
Yeast extract	3.0 g	Source of vitamins and other growth factors
Peptone	6.0 g	Source of amino acids, N, S, and P
Glucose	1.0 g	C and energy source
Agar	15.0 g	Inert solidifying agent
water	1000 ml	
pH 6.6		

Table 5b. Selective enrichment medium for growth of extreme halophiles.

Component	Amount	Function of component
Casamino acids	7.5 g	Source of amino acids, N, S and P
Yeast extract	10.0 g	Source of growth factors
Trisodium citrate	3.0 g	C and energy source
KCl	2.0 g	K^+ source
$MgSO_4$ 7 H_2O	20.0 g	S and Mg^{++} source
$FeCl_2$	0.023 g	Fe^{++} source
NaCl	250 g	Na^+ source for halophiles and inhibitory to nonhalophiles
water	1000 ml	
pH 7.4		

Physical and Environmental Requirements for Microbial Growth

The procaryotes exist in nature under an enormous range of physical conditions such as O_2 concentration, Hydrogen ion concentration (pH) and temperature. The exclusion limits of life on the planet, with regard to environmental parameters, are always set by some microorganism, most often a procaryote, and frequently an Archaeon. Applied to all microorganisms is a vocabulary of terms used to describe their growth (ability to grow) within a range of physical conditions. A thermophile grows at high temperatures, an acidophile grows at low pH, an osmophile grows at high solute concentration, and so on. This nomenclature will be employed in this

section to describe the response of the procaryotes to a variety of physical conditions.

The Effect of Oxygen

Oxygen is a universal component of cells and is always provided in large amounts by H_2O. However, procaryotes display a wide range of responses to molecular oxygen O_2 (Table 6).

Obligate aerobes require O_2 for growth; they use O_2 as a final electron acceptor in aerobic respiration.

Obligate anaerobes (occasionally called **aerophobes**) do not need or use O_2 as a nutrient. In fact, O_2 is a toxic substance, which either kills or inhibits their growth. Obligate anaerobic procaryotes may live by fermentation, anaerobic respiration, bacterial photosynthesis, or the novel process of methanogenesis.

Facultative anaerobes (or **facultative aerobes**) are organisms that can switch between aerobic and anaerobic types of metabolism. Under anaerobic conditions (no O_2) they grow by fermentation or anaerobic respiration, but in the presence of O_2 they switch to aerobic respiration.

Aerotolerant anaerobes are bacteria with an exclusively anaerobic (fermentative) type of metabolism but they are insensitive to the presence of O_2. They live by fermentation alone whether or not O_2 is present in their environment.

Table 6. Terms used to describe O_2 Relations of Microorganisms.

		Environment	
Group	**Aerobic**	**Anaerobic**	**O_2 Effect**
Obligate Aerobe	Growth	No growth	Required (utilized for aerobic respiration)
Microaerophile	Growth if level not too high	No growth	Required but at levels below 0.2 atm
Obligate Anaerobe	No growth	Growth Toxic	

Facultative Anaerobe (Facultative Aerobe)	Growth	Growth	Not required for growth but utilized when available
Aerotolerant Anaerobe	Growth	Growth	Not required and not utilized

The response of an organism to O_2 in its environment depends upon the occurrence and distribution of various enzymes which react with O_2 and various oxygen radicals that are invariably generated by cells in the presence of O_2. All cells contain enzymes capable of reacting with O_2. For example, oxidations of flavoproteins by O_2 invariably result in the formation of H_2O_2 (peroxide) as one major product and small quantities of an even more toxic free radical, superoxide or $O_2^{\cdot -}$. Also, chlorophyll and other pigments in cells can react with O_2 in the presence of light and generate singlet oxygen, another radical form of oxygen which is a potent oxidizing agent in biological systems.

In aerobes and aerotolerant anaerobes the potential for lethal accumulation of superoxide is prevented by the enzyme superoxide dismutase (Figure 1). All organisms which can live in the presence of O_2 (whether or not they utilize it in their metabolism) contain superoxide dismutase. Nearly all organisms contain the enzyme catalase, which decomposes H_2O_2. Even though certain aerotolerant bacteria such as the lactic acid bacteria lack catalase, they decompose H_2O_2 by means of peroxidase enzymes which derive electrons from $NADH_2$ to reduce peroxide to H_2O. Obligate anaerobes lack superoxide dismutase and catalase and/or peroxidase, and therefore undergo lethal oxidations by various oxygen radicals when they are exposed to O_2. See Figure 2 below.

All photosynthetic (and some nonphotosynthetic) organisms are protected from lethal oxidations of singlet oxygen by their possession of carotenoid pigments which physically react with the singlet oxygen radical and lower it to its nontoxic "ground" (triplet) state. Carotenoids are said to "quench" singlet oxygen radicals.

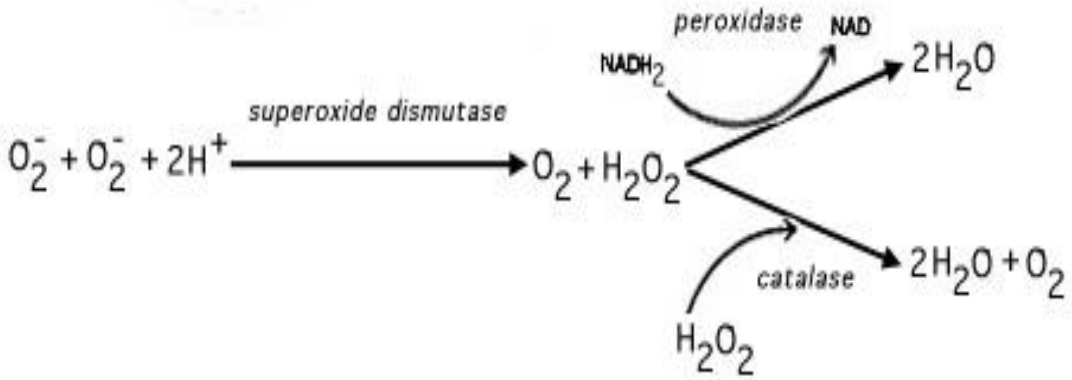


Figure 2. The action of superoxide dismutase, catalase and peroxidase. These enzymes detoxify oxygen radicals that are inevitably generated by living systems in the presence of O_2. The distribution of these enzymes in cells determines their ability to exist in the presence of O_2

Table 7. Distribution of superoxide dismutase, catalase and peroxidase in procaryotes with different O_2 tolerances.

Group	Superoxide dismutase	Catalase	Peroxidase
Obligate aerobes and most facultative anaerobes (e.g. Enterics)	+	+	-
Most aerotolerant anaerobes (e.g. Streptococci)	+	-	+
Obligate anaerobes (e.g. Clostridia, Methanogens, Bacteroides)	-	-	-

The Effect of pH on Growth

The pH, or hydrogen ion concentration, $[H^+]$, of natural environments varies from about 0.5 in the most acidic soils to about 10.5 in the most alkaline lakes. Appreciating that pH is measured on a logarithmic scale, the $[H^+]$ of natural environments varies over a billion-fold and some microorganisms are living at the extremes, as well as every point between the extremes! Most free-living procaryotes can grow over a range of 3 pH units, about a thousand fold change in $[H^+]$. The range of pH over which an organism grows is defined by **three cardinal points**: the **minimum pH**, below which the organism cannot grow, the **maximum pH**, above which the organism cannot grow, and the **optimum pH**, at which the organism

grows best. For most bacteria there is an orderly increase in growth rate between the minimum and the optimum and a corresponding orderly decrease in growth rate between the optimum and the maximum pH, reflecting the general effect of changing [H^+] on the rates of enzymatic reaction (Figure 3).

Microorganisms which grow at an optimum pH well below neutrality (7.0) are called **acidophiles**. Those which grow best at neutral pH are called **neutrophiles** and those that grow best under alkaline conditions are called **alkaliphiles**. Obligate acidophiles, such as some *Thiobacillus* species, actually require a low pH for growth since their membranes dissolve and the cells lyse at neutrality. Several genera of Archaea, including *Sulfolobus* and *Thermoplasma*, are obligate acidophiles. Among eukaryotes, many fungi are acidophiles, but the champion of growth at low pH is the eukaryotic alga *Cyanidium* which can grow at a pH of 0.

In the construction and use of culture media, one must always consider the optimum pH for growth of a desired organism and incorporate **buffers** in order to maintain the pH of the medium in the changing milieu of bacterial waste products that accumulate during growth. Many pathogenic bacteria exhibit a relatively narrow range of pH over which they will grow. Most diagnostic media for the growth and identification of human pathogens have a pH near 7.

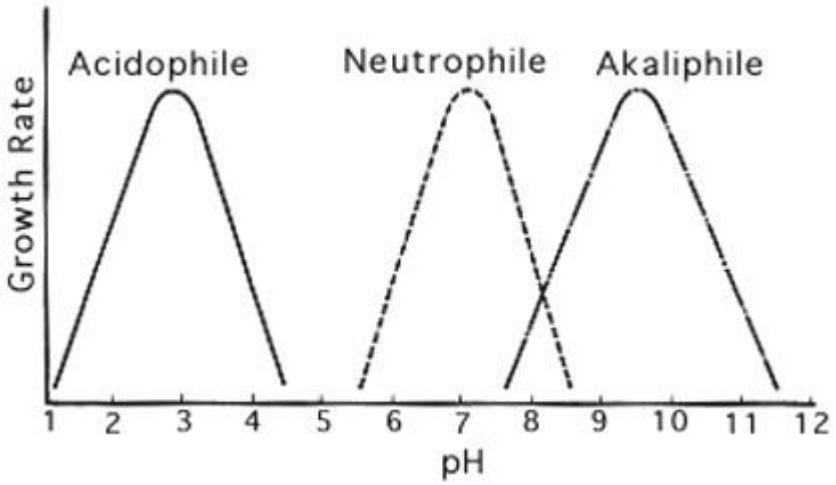


Figure 3. Growth rate vs pH for three environmental classes of procaryotes. Most free-living bacteria grow over a pH range of about three units. Note the symmetry of the curves below and above the optimum pH for growth.

Table 8. Minimum, maximum and optimum pH for growth of certain procaryotes.

Organism	Minimum pH	Optimum pH	Maximum pH
Thiobacillus thiooxidans	0.5	2.0-2.8	4.0-6.0
Sulfolobus acidocaldarius	1.0	2.0-3.0	5.0
Bacillus acidocaldarius	2.0	4.0	6.0
Zymomonas lindneri	3.5	5.5-6.0	7.5
Lactobacillus acidophilus	4.0-4.6	5.8-6.6	6.8
Staphylococcus aureus	4.2	7.0-7.5	9.3
Escherichia coli	4.4	6.0-7.0	9.0
Clostridium sporogenes	5.0-5.8	6.0-7.6	8.5-9.0
Erwinia caratovora	5.6	7.1	9.3
Pseudomonas aeruginosa	5.6	6.6-7.0	8.0
Thiobacillus novellus	5.7	7.0	9.0
Streptococcus pneumoniae	6.5	7.8	8.3
Nitrobacter sp	6.6	7.6-8.6	10.0

The Effect of Temperature on Growth

Microorganisms have been found growing in virtually all environments where there is liquid water, regardless of its temperature. In 1966, Professor Thomas D. Brock, then at Indiana University, made the amazing discovery in boiling hot springs of Yellowstone National Park that bacteria were not just surviving there, they were growing and flourishing.

Subsequently, procaryotes have been detected growing around black smokers and hydrothermal vents in the deep sea at temperatures at least as high as 120 degrees. Microorganisms have been found growing at very low temperatures as well. In supercooled solutions of H_2O as low as -20 degrees, certain organisms can extract water for growth, and many forms of life flourish in the icy waters of the Antarctic, as well as household refrigerators, near 0 degrees.

A particular microorganism will exhibit a range of temperature over which it can grow, defined by three cardinal points in the same manner as pH (Figure 5, cf. Figure 3). Considering the total span of temperature where liquid water exists, the procaryotes may be subdivided into several subclasses on the basis of one or another of their cardinal points for growth. For example, organisms with an optimum temperature near 37 degrees (the body temperature of warm-blooded animals) are called **mesophiles**. Organisms with an optimum T between about 45 degrees and 70 degrees are **thermophiles**. Some Archaea with an optimum T of 80 degrees or higher and a maximum T as high as 115 degrees, are now referred to as **extreme thermophiles** or **hyperthermophiles**. The cold-loving organisms are **psychrophiles** defined by their ability to grow at 0 degrees. A variant of a psychrophile (which usually has an optimum T of 10-15 degrees) is a **psychrotroph**, which grows at 0 degrees but displays an optimum T in the mesophile range, nearer room temperature. Psychrotrophs are the scourge of food storage in refrigerators since they are invariably brought in from their mesophilic habitats and continue to grow in the refrigerated environment where they spoil the food. Of course, they grow slower at 2 degrees than at 25 degrees. Think how fast milk spoils on the counter top versus in the refrigerator.

Psychrophilic bacteria are adapted to their cool environment by having largely unsaturated fatty acids in their plasma membranes. Some psychrophiles, particularly those from the Antarctic have been found to contain polyunsaturated fatty acids, which generally do not occur in procaryotes. The degree of unsaturation of a fatty acid correlates with its solidification T or thermal transition stage (i.e., the temperature at which the lipid melts or solidifies); unsaturated fatty acids remain liquid at low T but are also denatured at moderate T; saturated fatty acids, as in the membranes of thermophilic bacteria, are stable at high temperatures, but they also solidify at relatively high T. Thus, saturated fatty acids (like butter) are solid at room temperature while unsaturated fatty acids (like safflower oil) remain liquid in the refrigerator. Whether fatty acids in a membrane are in a liquid or a solid phase affects the fluidity of the membrane, which directly affects its ability to function. Psychrophiles also have enzymes that continue to function, albeit at a reduced rate,

at temperatures at or near 0 degrees. Usually, psychrophile proteins and/or membranes, which adapt them to low temperatures, do not function at the body temperatures of warm-blooded animals (37 degrees) so that they are unable to grow at even moderate temperatures.

Thermophiles are adapted to temperatures above 60 degrees in a variety of ways. Often thermophiles have a high G + C content in their DNA such that the melting point of the DNA (the temperature at which the strands of the double helix separate) is at least as high as the organism's maximum T for growth. But this is not always the case, and the correlation is far from perfect, so thermophile DNA must be stabilized in these cells by other means. The membrane fatty acids of thermophilic bacteria are highly saturated allowing their membranes to remain stable and functional at high temperatures. The membranes of hyperthermophiles, virtually all of which are Archaea, are not composed of fatty acids but of repeating subunits of the C5 compound, phytane, a branched, saturated, "isoprenoid" substance, which contributes heavily to the ability of these bacteria to live in superheated environments. The structural proteins (e.g. ribosomal proteins, transport proteins (permeases) and enzymes of thermophiles and hyperthermophiles are very heat stable compared with their mesophilic counterparts. The proteins are modified in a number of ways including dehydration and through slight changes in their primary structure, which accounts for their thermal stability.

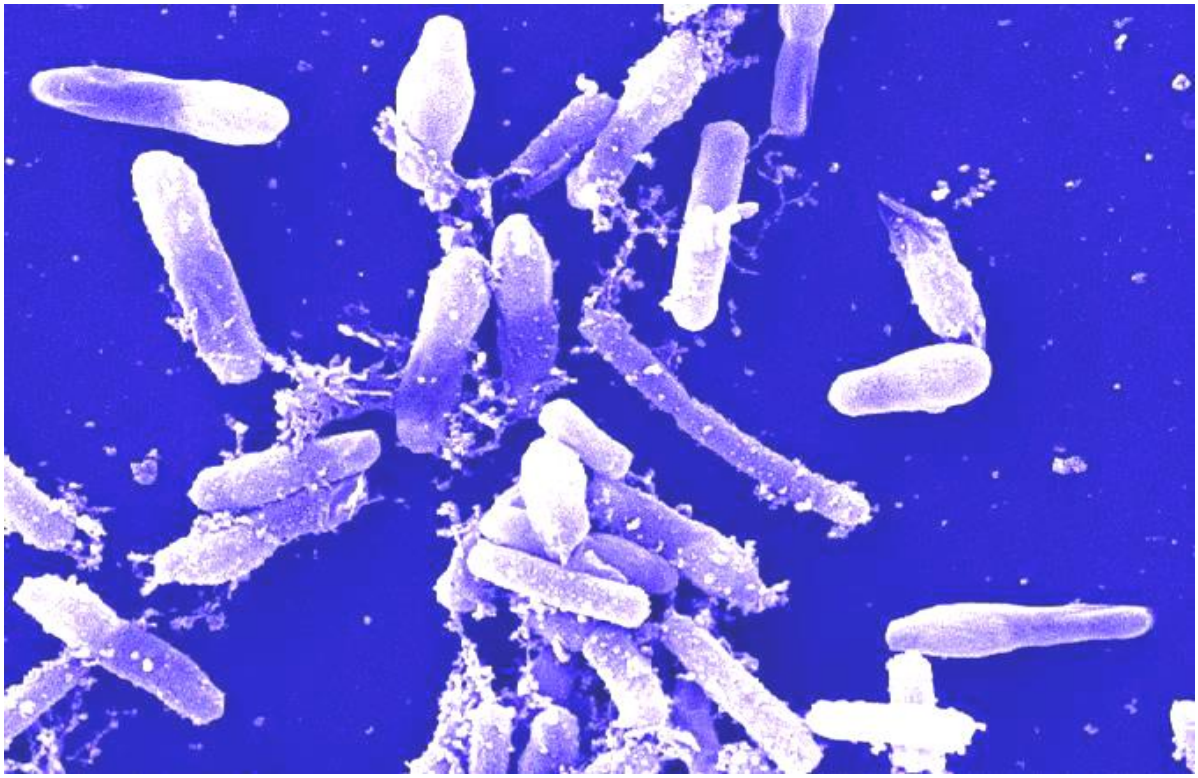


Figure 4. SEM of a thermophilic *Bacillus* species isolated from a compost pile at 55° C. The rods are 3-5 microns in length and 0.5 to 1 micron in width with terminal endospores in a slightly-swollen sporangium.

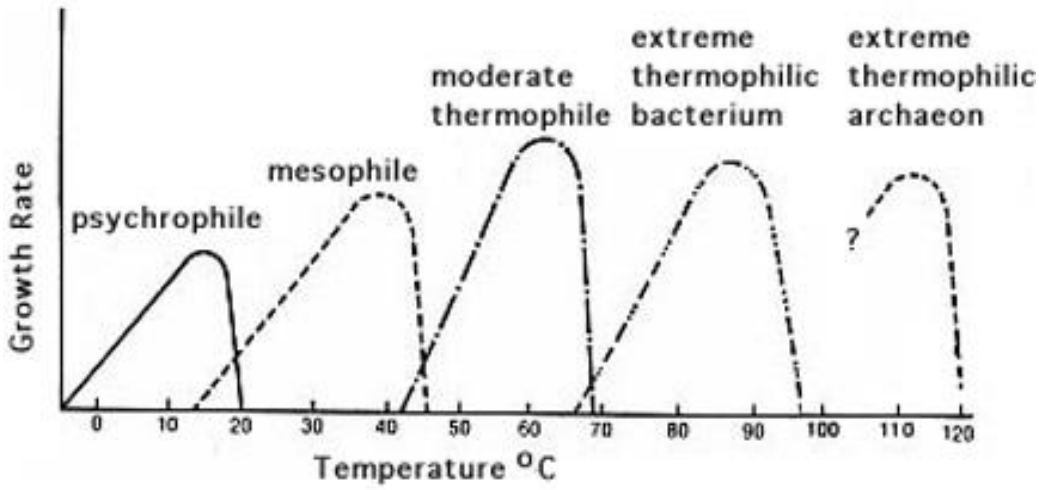


Figure 5. Growth rate vs temperature for five environmental classes of procaryotes. Most procaryotes will grow over a temperature range of about 30 degrees. The curves exhibit three cardinal points: minimum, optimum and maximum temperatures for growth. There is a steady increase in growth rate between the minimum and optimum temperatures, but slightly past the optimum a critical thermolabile cellular event occurs, and the growth rates plunge rapidly as the maximum T is approached. As expected and as predicted by

T.D. Brock, life on earth, with regard to temperature, exists wherever water remains in a liquid state. Thus, psychrophiles grow in solution wherever water is supercooled below 0 degrees; and extreme thermophilic archaea (hyperthermophiles) have been identified growing near deep-sea thermal vents at temperatures up to 120 degrees. Theoretically, the bar can be pushed to even higher temperatures.

Table 9. Terms used to describe microorganisms in relation to temperature requirements for growth.

Temperature for growth (degrees C)

Group	Minimum	Optimum	Maximum	Comments
Psychrophile	Below 0	10-15	Below 20	Grow best at relatively low T
Psychrotroph	0	15-30	Above 25	Able to grow at low T but prefer moderate T
Mesophile	10-15	30-40	Below 45	Most bacteria esp. those living in association with warm-blooded animals
Thermophile*	45	50-85	Above 100 (boiling)	Among all thermophiles is wide variation in optimum and maximum T

*For "degrees" of thermophily see text and graphs above

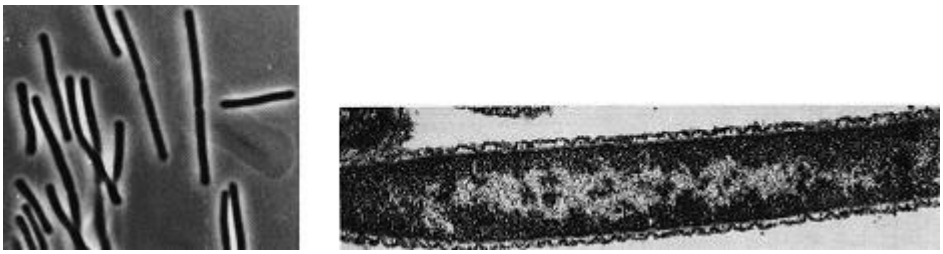


Figure 6. *Thermus aquaticus,* the thermophilic bacterium that is the source of taq polymerase. L wet mount; R electron micrograph.

Table 10. Minimum, maximum and optimum temperature for growth of certain bacteria and archaea.

Temperature for growth (degrees C)

Bacterium	Minimum	Optimum	Maximum
Listeria monocytogenes	1	30-37	45
Vibrio marinus	4	15	30
Pseudomonas maltophilia	4	35	41
Thiobacillus novellus	5	25-30	42
Staphylococcus aureus	10	30-37	45
Escherichia coli	10	37	45
Clostridium kluyveri	19	35	37
Streptococcus pyogenes	20	37	40
Streptococcus pneumoniae	25	37	42
Bacillus flavothermus	30	60	72
Thermus aquaticus	40	70-72	79
Methanococcus jannaschii	60	85	90
Sulfolobus acidocaldarius	70	75-85	90
Pyrobacterium brockii	80	102-105	115

Water Availability

Water is the solvent in which the molecules of life are dissolved, and the availability of water is therefore a critical factor that affects the growth of all cells. The availability of water for a cell depends upon its presence in the atmosphere (relative humidity) or its presence in solution or a substance (**water activity**). The water activity (A_w) of pure H_2O is 1.0 (100% water). Water activity is affected by the presence of solutes such as salts or sugars, that are dissolved in the water. The higher the solute concentration of a substance, the lower is the water activity and vice-versa. Microorganisms live over a range of A_w from 1.0 to 0.7. The A_w of human blood is 0.99; seawater = 0.98; maple syrup = 0.90; Great Salt Lake = 0.75. Water activities in agricultural soils range between 0.9 and 1.0.

The only common solute in nature that occurs over a wide concentration range is salt [NaCl], and some microorganisms are named based on their growth response to salt. Microorganisms that require some NaCl for growth are **halophiles**. **Mild halophiles** require 1-6% salt, **moderate halophiles** require 6-15% salt; **extreme halophiles** that require 15-30% NaCl for growth are found among the archaea. Bacteria that are able to grow at moderate salt concentrations, even though they grow best in the absence of NaCl, are called **halotolerant**. Although halophiles are "osmophiles" (and halotolerant organisms are "osmotolerant") the term **osmophiles** is usually reserved for organisms that are able to live in environments high in sugar. Organisms which live in dry environments (made dry by lack of water) are called **xerophiles**.

The concept of lowering water activity in order to prevent bacterial growth is the basis for preservation of foods by drying (in sunlight or by evaporation) or by addition of high concentrations of salt or sugar.

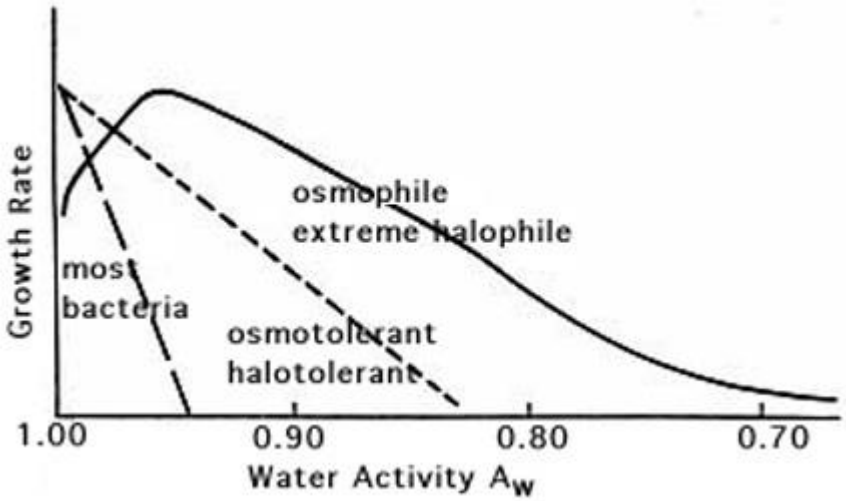


Figure 7. Growth rate vs osmolarity for different classes of procaryotes. Osmolarity is determined by solute concentration in the environment. Osmolarity is inversely related to water activity (A_w), which is more like a measure of the concentration of water (H_2O) in a solution. Increased solute concentration means increased osmolarity and decreased A_w. TFrom left to right the graph shows the growth rate of a normal (nonhalophile) such as *E. coli* or *Pseudomonas,* the growth rate of a halotolerant bacterium such as *Staphylococcus aureus,* and the growth rate of an extreme halophile such as the archaean *Halococcus.*Note that a true halophile grows best at salt concentrations where most bacteria are inhibited.

GROWTH OF BACTERIAL POPULATIONS

Measurement of Bacterial Growth

Growth is an orderly increase in the quantity of cellular constituents. It depends upon the ability of the cell to form new protoplasm from nutrients available in the environment. In most bacteria, growth involves increase in cell mass and number of ribosomes, duplication of the bacterial chromosome, synthesis of new cell wall and plasma membrane, partitioning of the two chromosomes, septum formation, and cell division. This asexual process of reproduction is called **binary fission**.

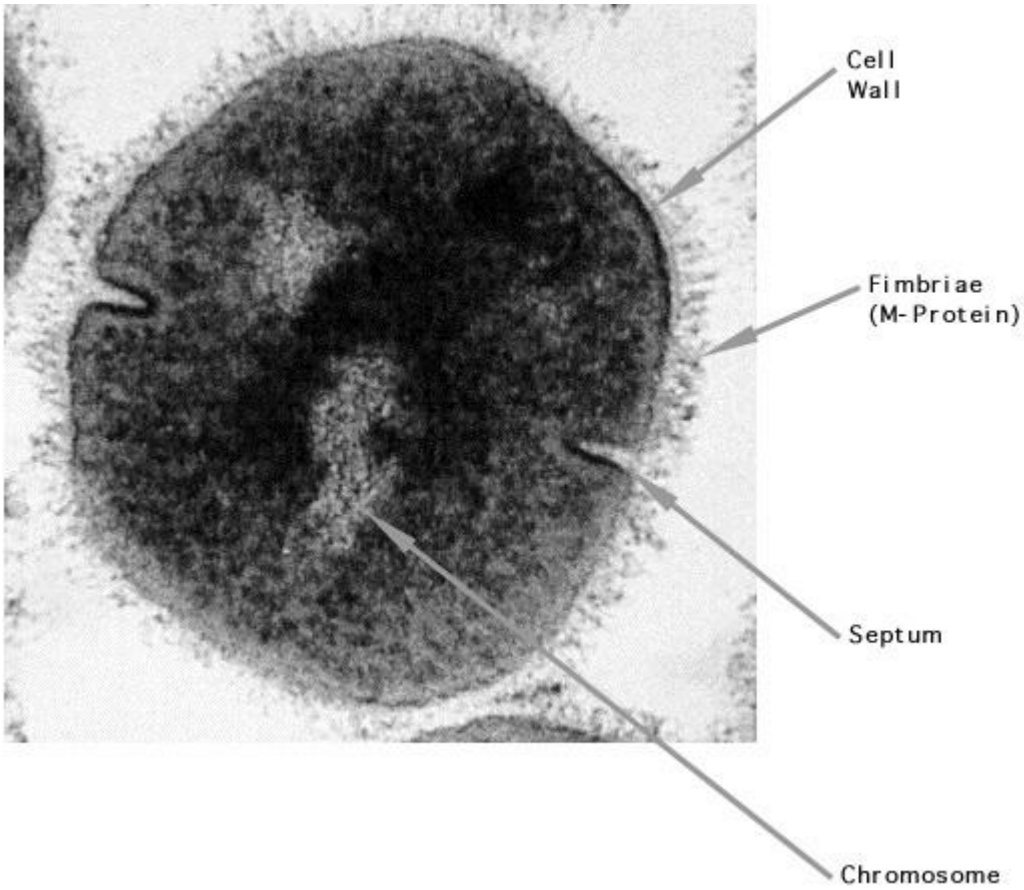


Figure 1. Bacterial growth by binary fission. Most bacteria reproduce by a relatively simple asexual process called binary fission: each cell increases in size and divides into two cells. During this process there is an orderly increase in cellular structures and components, replication and segregation of the bacterial DNA, and formation of a septum or cross wall which divides the cell into two progeny cells. The process is coordinated by the bacterial membrane perhaps by means of mesosomes. The DNA molecule is believed to be attached to a point on

the membrane where it is replicated. The two DNA molecules remain attached at points side-by-side on the membrane while new membrane material is synthesized between the two points. This draws the DNA molecules in opposite directions while new cell wall and membrane are laid down as a septum between the two chromosomal compartments. When septum formation is complete the cell splits into two progeny cells. The time interval required for a bacterial cell to divide or for a population of bacterial cells to double is called the generation time. Generation times for bacterial species growing in nature may be as short as 15 minutes or as long as several days. Electron micrograph of *Streptococcus pyogenes*.

For unicellular organisms such as the bacteria, growth can be measured in terms of two different parameters: changes in **cell mass** and changes in **cell numbers**.

Methods for Measurement of Cell Mass

Methods for measurement of the cell mass involve both direct and indirect techniques.

1. Direct **physical measurement** of dry weight, wet weight, or volume of cells after centrifugation.

2. Direct **chemical measurement** of some chemical component of the cells such as total N, total protein, or total DNA content.

3. Indirect **measurement of chemical activity** such as rate of O_2 production or consumption, CO_2 production or consumption, etc.

4. **Turbidity measurements** employ a variety of instruments to determine the amount of light scattered by a suspension of cells. Particulate objects such as bacteria scatter light in proportion to their numbers. The turbidity or **optical density** of a suspension of cells is directly related to cell mass or cell number, after construction and calibration of a standard curve. The method is simple and nondestructive, but the sensitivity is limited to about 10^7 cells per ml for most bacteria.

Methods for Measurement of Cell Numbers

Measuring techniques involve direct counts, visually or instrumentally, and indirect viable cell counts.

1. **Direct microscopic counts** are possible using special slides known as counting chambers. Dead cells cannot be distinguished from living ones. Only dense suspensions can be counted (>10^7 cells per ml), but samples can be concentrated by centrifugation or filtration to increase sensitivity.

A variation of the direct microscopic count has been used to observe and measure growth of bacteria in natural environments. In order to detect and prove that thermophilic bacteria were growing in boiling hot springs, T.D. Brock immersed microscope slides in the springs and withdrew them periodically for microscopic observation. The bacteria in the boiling water attached to the glass slides naturally and grew as microcolonies on the surface.

2. **Electronic counting chambers** count numbers and measure size distribution of cells. For cells the size of bacteria the suspening medium must be very clean. Such elecronic devices are more often used to count eukaryotic cells such as blood cells.

3. **Indirect viable cell counts**, also called **plate counts**, involve plating out (spreading) a sample of a culture on a nutrient agar surface. The sample or cell suspension can be diluted in a nontoxic diluent (e.g. water or saline) before plating. If plated on a suitable medium, each viable unit grows and forms a colony. Each colony that can be counted is called a **colony forming unit (cfu)** and the number of cfu's is related to the viable number of bacteria in the sample.

Advantages of the technique are its sensitivity (theoretically, a single cell can be detected), and it allows for inspection and positive identification of the organism counted. Disadvantages are (1) only living cells develop colonies that are counted; (2) clumps or chains of cells develop into a single colony; (3) colonies develop only from those organisms for which the cultural conditions are suitable for growth. The latter makes the technique virtually useless to characterize or count the **total**

number of bacteria in complex microbial ecosystems such as soil or the animal rumen or gastrointestinal tract. Genetic probes can be used to demonstrate the diversity and relative abundance of procaryotes in such an environment, but many species identified by genetic techniques have so far proven unculturable.

Table 1. Some Methods used to measure bacterial growth

Method	Application	Comments
Direct microscopic count	Enumeration of bacteria in milk or cellular vaccines	Cannot distinguish living from nonliving cells
Viable cell count (colony counts)	Enumeration of bacteria in milk, foods, soil, water, laboratory cultures, etc.	Very sensitive if plating conditions are optimal
Turbidity measurement	Estimations of large numbers of bacteria in clear liquid media and broths	Fast and nondestructive, but cannot detect cell densities less than 10^7 cells per ml
Measurement of total N or protein	Measurement of total cell yield from very dense cultures	only practical application is in the research laboratory
Measurement of Biochemical activity e.g. O2 uptake CO2 production, ATP production, etc.	Microbiological assays	Requires a fixed standard to relate chemical activity to cell mass and/or cell numbers
Measurement of dry weight or wet weight of cells or volume of cells after centrifugation	Measurement of total cell yield in cultures	probably more sensitive than total N or total protein measurements

The Bacterial Growth Curve

In the laboratory, under favorable conditions, a growing bacterial population doubles at regular intervals. Growth is by geometric progression: 1, 2, 4, 8, etc. or 2^0, 2^1, 2^2, 2^3.....2^n (where n = the number of generations). This is called **exponential growth**. In reality, exponential growth is only part of the bacterial life cycle, and not representative of the normal pattern of growth of bacteria in Nature.

When a fresh medium is inoculated with a given number of cells, and the population growth is monitored over a period of time, plotting the data will yield a **typical bacterial growth curve** (Figure 2).

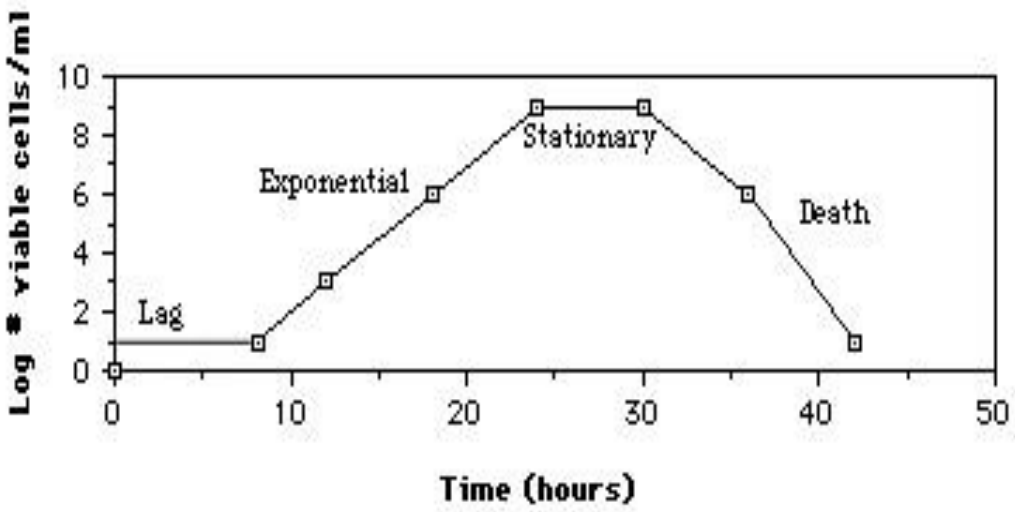


Figure 2. The typical bacterial growth curve. When bacteria are grown in a closed system (also called a batch culture), like a test tube, the population of cells almost always exhibits these growth dynamics: cells initially adjust to the new medium (lag phase) until they can start dividing regularly by the process of binary fission (exponential phase). When their growth becomes limited, the cells stop dividing (stationary phase), until eventually they show loss of viability (death phase). Note the parameters of the x and y axes. Growth is expressed as change in the number viable cells vs time. Generation times are calculated during the exponential phase of growth. Time measurements are in hours for bacteria with short generation times.

Four characteristic phases of the growth cycle are recognized.

1. **Lag Phase**. Immediately after inoculation of the cells into fresh medium, the population remains temporarily unchanged. Although there is no apparent cell division occurring, the cells may be growing in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in metabolic activity.

The length of the lag phase is apparently dependent on a wide variety of factors including the size of the inoculum; time necessary to recover from physiacal damage or shock in the transfer; time required for synthesis of essential coenzymes or

division factors; and time required for synthesis of new (inducible) enzymes that are necessary to metabolize the substrates present in the medium.

2. **Exponential (log) Phase**. The exponential phase of growth is a pattern of balanced growth wherein all the cells are dividing regularly by binary fission, and are growing by geometric progression. The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation. The rate of exponential growth of a bacterial culture is expressed as **generation time**, also the **doubling time** of the bacterial population. Generation time (G) is defined as the time (t) per generation (n = number of generations). Hence, G=t/n is the equation from which calculations of generation time (below) derive.

3. **Stationary Phase**. Exponential growth cannot be continued forever in a **batch culture** (e.g. a closed system such as a test tube or flask). Population growth is limited by one of three factors: 1. exhaustion of available nutrients; 2. accumulation of inhibitory metabolites or end products; 3. exhaustion of space, in this case called a lack of "biological space".

During the stationary phase, if viable cells are being counted, it cannot be determined whether some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing. The stationary phase, like the lag phase, is not necessarily a period of quiescence. Bacteria that produce **secondary metabolites**, such as antibiotics, do so during the stationary phase of the growth cycle (Secondary metabolites are defined as metabolites produced after the active stage of growth). It si during the stationary phase that spore-forming bacteria have to induce or unmask the activity of dozens of genes that may be involved in sporulation process.

4. **Death Phase**. If incubation continues after the population reaches stationary phase, a death phase follows, in which the viable cell population declines. (Note, if counting by turbidimetric measurements or microoscopic counts, the death phase cannot be observed.). During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase.

Growth Rate and Generation Time

As mentioned above, bacterial growth rates during the phase of exponential growth, under standard nutritional conditions (culture medium, temperature, pH, etc.), define the bacterium's generation time. Generation times for bacteria vary from about 12 minutes to 24 hours or more. The generation time for *E. coli* in the laboratory is 15-20 minutes, but in the intestinal tract, the coliform's generation time is estimated to be 12-24 hours. For most known bacteria that can be cultured, generation times range from about 15 minutes to 1 hour. Symbionts such as *Rhizobium* tend to have longer generation times. Many lithotrophs, such as the nitrifying bacteria, also have long generation times. Some bacteria that are pathogens, such as *Mycobacterium tuberculosis* and *Treponema pallidum*, have especially long generation times, and this is thought to be an advantage in their virulence. Generation times for a few bacteria are are shown in Table 2.

Table 2. Generation times for some common bacteria under optimal conditions of growth.

Bacterium	Medium	Generation Time (minutes)
Escherichia coli	Glucose-salts	17
Bacillus megaterium	Sucrose-salts	25
Streptococcus lactis	Milk	26
Streptococcus lactis	Lactose broth	48
Staphylococcus aureus	Heart infusion broth	27-30
Lactobacillus acidophilus	Milk	66-87
Rhizobium japonicum	Mannitol-salts-yeast extract	344-461
Mycobacterium tuberculosis	Synthetic	792-932
Treponema pallidum	Rabbit testes	1980

Continuous Culture of Bacteria

The cultures so far discussed for growth of bacterial populations are called **batch cultures**. Since the nutrients are not renewed, exponential growth is limited to a few generations. Bacterial cultures can be maintained in a state of exponential growth over long periods of time using a system of **continuous culture** (Figure 3), designed to relieve the conditions that stop exponential growth in batch cultures. Continuous culture, in a device called a **chemostat**, can be used to maintain a bacterial population at a constant density, a situation that is, in many ways, more similar to bacterial growth in natural environments.

In a chemostat, the growth chamber is connected to a reservoir of sterile medium. Once growth is initiated, fresh medium is continuously supplied from the reservoir. The volume of fluid in the growth chamber is maintained at a constant level by some sort of overflow drain. Fresh medium is allowed to enter into the growth chamber at a rate that limits the growth of the bacteria. The bacteria grow (cells are formed) at the same rate that bacterial cells (and spent medium) are removed by the overflow. The rate of addition of the fresh medium determines the rate of growth because the fresh medium always contains a limiting amount of an essential nutrient. Thus, the chemostat relieves the insufficiency of nutrients, the accumulation of toxic substances, and the accumulation of excess cells in the culture, which are the parameters that initiate the stationary phase of the growth cycle. The bacterial culture can be grown and maintained at relatively constant conditions, depending on the flow rate of the nutrients.

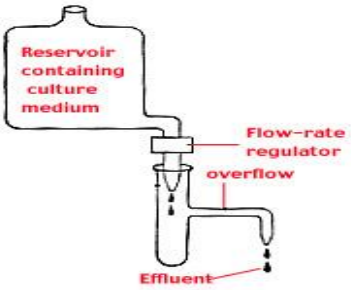


Figure 3. Schematic diagram of a chemostat, a device for the continuous culture of bacteria. The chemostat relieves the environmental conditions that restrict growth by continuously supplying nutrients to cells and removing waste substances and spent cells from the culture medium.

Synchronous Growth of Bacteria

Studying the growth of bacterial populations in batch or continuous cultures does not permit any conclusions about the growth behavior of individual cells, because the distribution of cell size (and hence cell age) among the members of the population is completely random. Information about the growth behavior of individual bacteria can, however, be obtained by the study of **synchronous cultures**. Synchronized cultures must be composed of cells which are all at the same stage of the **bacterial cell cycle**. Measurements made on synchronized cultures are equivalent to measurements made on individual cells.

A number of clever techniques have been devised to obtain bacterial populations at the same stage in the cell cycle. Some techniques involve manipulation of environmental parameters which induces the population to start or stop growth at the same point in the cell cycle, while others are physical methods for selection of cells that have just completed the process of binary fission. Theoretically, the smallest cells in a bacterial population are those that have just completed the process of cell division. Synchronous growth of a population of bacterial cells is illustrated in Figure 5. Synchronous cultures rapidly lose synchrony because not all cells in the population divide at exactly the same size, age or time.

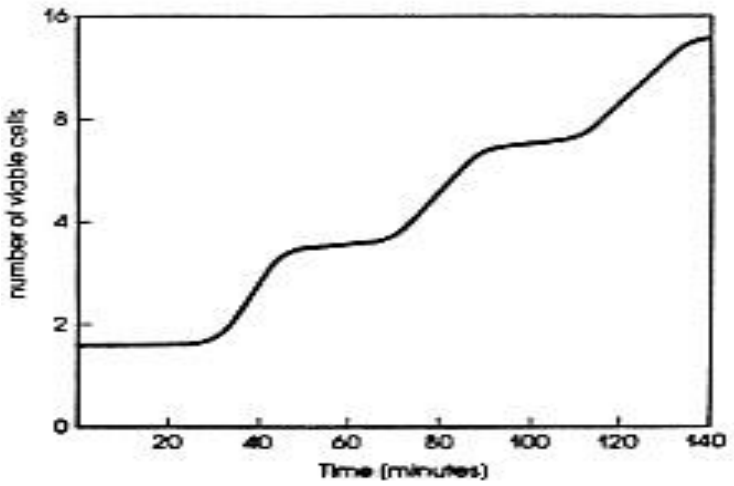


Figure 4. The synchronous growth of a bacterial population. By careful selection of cells that have just divided, a bacterial population can be synchronized in the bacterial cell division cycle. Synchrony can be maintained for only a few generations.

THE CONTROL OF MICROBIAL GROWTH

Introduction

The control of microbial growth is necessary in many practical situations, and significant advances in agriculture, medicine, and food science have been made through study of this area of microbiology.

"Control of growth", as used here, means to prevent growth of microorganisms. This control is affected in two basic ways: (1) by killing microorganisms or (2) by inhibiting the growth of microorganisms. Control of growth usually involves the use of physical or chemical agents which either kill or prevent the growth of microorganisms. Agents which kill cells are called **cidal** agents; agents which inhibit the growth of cells (without killing them) are referred to as **static** agents. Thus the term **bactericidal** refers to killing bacteria and **bacteriostatic** refers to inhibiting the growth of bacterial cells. A **bactericide** kills bacteria, a **fungicide** kills fungi, and so on.

Sterilization is the complete destruction or elimination of all viable organisms (in or on an object being sterilized). There are no degrees of sterilization: an object is

either sterile or not. Sterilization procedures involve the use of heat, radiation or chemicals, or physical removal of cells.

Methods of Sterilization

Heat: most important and widely used. For sterilization always consider type of heat, time of application and temperature to ensure destruction of all microorganisms. Endospores of bacteria are considered the most thermoduric of all cells so their destruction guarantees sterility.

Incineration: burns organisms and physically destroys them. Used for needles , inoculating wires, glassware, etc. and objects not destroyed in the incineration process.

Boiling: 100° for 30 minutes. Kills everything except some endospores (Actually, for the purposes of purifying drinking water 100° for five minutes is probably adequate though there have been some reports that Giardia cysts can survive this process). To kill endospores, and therefore **sterilize** the solution, very long or **intermittent boiling** is required.

Autoclaving (steam under pressure or pressure cooker): 121° for 15 minutes (15#/in^2 pressure). Good for sterilizing almost anything, but heat-labile substances will be denatured or destroyed.

Dry heat (hot air oven): 160°/2hours or 170°/1hour. Used for glassware, metal, and objects that won't melt.

The protocol and recommendations for the use of heat to control microbial growth are given in Table 1.

Table 1. Recommended use of heat to control bacterial growth

Treatment	Temperature	Effectiveness
Incineration	>500°	Vaporizes organic material on nonflammable surfaces but may destroy many substances in the process

Boiling	100°	30 minutes of boiling kills microbial pathogens and vegetative forms of bacteria but may not kill bacterial endospores
Intermittent boiling	100°	Three 30-minute intervals of boiling, followed by periods of cooling kills bacterial endospores
Autoclave and pressure cooker (steam under pressure)	121°/15 minutes at 15# pressure	kills all forms of life including bacterial endospores. The substance being sterilized must be maintained at the effective T for the full time
Dry heat (hot air oven)	160°/2 hours	For materials that must remain dry and which are not destroyed at T between 121° and 170° Good for glassware, metal, not plastic or rubber items
Dry heat (hot air oven)	170°/1 hour	Same as above. Note increasing T by 10 degrees shortens the sterilizing time by 50 percent
Pasteurization (batch method)	63°/30 minutes	kills most vegetative bacterial cells including pathogens such as streptococci, staphylococci and Mycobacterium tuberculosis
Pasteurization (flash method)	72°/15 seconds	Effect on bacterial cells similar to batch method; for milk, this method is more conducive to industry and has fewer undesirable effects on quality or taste

Irradiation: usually destroys or distorts nucleic acids. Ultraviolet light is usually used (commonly used to sterilize the surfaces of objects), although x-rays and microwaves are possibly useful.

Filtration: involvres the physical removal (exclusion) of all cells in a liquid or gas, especially important to sterilize solutions which would be denatured by heat (e.g. antibiotics, injectable drugs, amino acids, vitamins, etc.)

Chemical and gas: (formaldehyde, glutaraldehyde, ethylene oxide) toxic chemicals kill all forms of life in a specialized gas chamber.

Control of Microbial Growth by Physical Agents

Applications of Heat The lethal **temperature** varies in microorganisms. The **time** required to kill depends on the number of organisms, species, nature of the product

being heated, pH, and temperature. Whenever heat is used to control microbial growth inevitably **both time and temperature are considered**.

Sterilization (boiling, autoclaving, hot air oven) kills all microorganisms with heat; commonly employed in canning, bottling, and other sterile packaging procedures.

Pasteurization is the use of mild heat to reduce the number of microorganisms in a product or food. In the case of pasteurization of milk the time and temperature depend on killing potential pathogens that are transmitted in milk, i.e., staphylococci, streptococci, Brucella abortus and Mycobacterium tuberculosis. For pasteurzation of milk: batch nethod: 63°/30minutes; flash method: 71°/15 seconds.

Low temperature (refrigeration and freezing): Most organisms grow very little or not at all at 0o. Store perishable foods at low temperatues to slow rate of growth and consequent spoilage (e.g. milk). Low temperatures are not bactericidal. Psychrotrophs, rather than true psychrophiles, are the usual cause of food spoilage in refrigerated foods.

Drying (removal of H_2O): Most microorganisms cannot grow at reduced water activity (Aw < 0.90). Often used to preserve foods (e.g. fruits, grains, etc.). Methods involve removal of water from product by heat, evaporation, freeze-drying, addition of salt or sugar.

Irradiation (microwave, UV, x-ray): destroys microorganisms as described under "sterilization". Many spoilage organisms are easily killed by irradiation. In some parts of Europe, fruits and vegetables are irradiated to increase their shelf life up to 500 percent. The practice has not been accepted in the U.S.

Control of microbial growth by chemical agents

Antimicrobial agents are chemicals that kill or inhibit the growth microorganisms. Antimicrobial agents include chemical preservatives and antiseptics, as well as drugs used in the treatment of infectious diseases of plants and animals. Antimicrobial agents may be of natural or synthetic origin, and they may have a static or cidal effect on microorganisms.

Types of antimicrobial agents

Antiseptics: microbicidal agents harmless enough to be applied to the skin and mucous membrane; should not be taken internaslly. Examples: mercurials, silver nitrate, iodine solution, alcohols, detergents.

Disinfectants: Agents that kill microorganisms, but not necessarily their spores,not safe for application to living tissues; they are used on inanimate objects such as tables, floors, utensils, etc. Examples: chlorine, hypochlorites, chlorine compounds, lye, copper sulfate, quaternary ammonium compounds.

Note: disinfectants and antiseptics are distinguished on the basis of whether they are safe for application to mucous membranes. Often, safety depends on the concentration of the compound. For example, sodium hypochlorite (chlorine), as added to water is safe for drinking, but "chlorox" (5% hypochlorite), an excellent disinfectant, is hardly safe to drink.

Common antiseptics and disinfectants and their uses are summarized in Table 2.

Table 2. Common antiseptics and disinfectants

Chemical	Action	Uses
Ethanol (50-70%)	Denatures proteins and solubilizes lipids	Antiseptic used on skin
Isopropanol (50-70%)	Denatures proteins and solubilizes lipids	Antiseptic used on skin
Formaldehyde (8%)	Reacts with NH_2, SH and COOH groups	Disinfectant, kills endospores
Tincture of Iodine (2% I2 in 70% alcohol)	Inactivates proteins	Antiseptic used on skin
Chlorine (Cl_2) gas	Forms hypochlorous acid (HClO), a strong oxidizing agent	Disinfect drinking water; general disinfectant
Silver nitrate ($AgNO_3$)	Precipitates proteins	General antiseptic and used in the eyes of newborns

Mercuric chloride	Inactivates proteins by reacting with sulfide groups	Disinfectant, although occasionally used as an antiseptic on skin
Detergents (e.g. quaternary ammonium compounds)	Disrupts cell membranes	Skin antiseptics and disinfectants
Phenolic compounds (e.g. carboloic acid, lysol, hexylresorcinol, hexachlorophene)	Denature proteins and disrupt cell membranes	Antiseptics at low concentrations; disinfectants at high concentrations
Ethylene oxide gas	Alkylating agent	Disinfectant used to sterilize heat-sensitive objects such as rubber and plastics

Preservatives: static agents used to inhibit the growth of microorganisms, most often in foods. If eaten they should be nontoxic. Examples; calcium propionate, sodium benzoate, formaldehyde, nitrate, sulfur dioxide. Table 3 is a list of common preservative and their uses.

Table 3. Common food preservatives and their uses

Preservative	Effective Concentration	Uses
Propionic acid and propionates	0.32%	Antifungal agent in breads, cake, Swiss cheeses
Sorbic acid and sorbates	0.2%	Antifungal agent in cheeses, jellies, syrups, cakes
Benzoic acid and benzoates	0.1%	Antifungal agent in margarine, cider, relishes, soft drinks
Sodium diacetate	0.32%	Antifungal agent in breads
Lactic acid	unknown	Antimicrobial agent in cheeses, buttermilk, yogurt and pickled foods
Sulfur dioxide, sulfites	200-300 ppm	Antimicrobial agent in dried fruits, grapes, molasses
Sodium nitrite	200 ppm	Antibacterial agent in cured meats, fish
Sodium chloride	unknown	Prevents microbial spoilage of meats, fish, etc.
Sugar	unknown	Prevents microbial spoilage of preserves, jams,

		syrups, jellies, etc.
Wood smoke	unknown	Prevents microbial spoilage of meats, fish, etc.

Chemotherapeutic agents: antimicrobial agents of synthetic origin useful in the treatment of microbial or viral disease. Examples: sulfonilamides, isoniazid, ethambutol, AZT, chloramphenicol. Note that the microbiologist's definition of a chemotherapeutic agent requires that the agent be used for antimicrobial purposes and so excludes synthetic agents used for therapy against diseases that are not of microbial origin.

Antibiotics: antimicrobial agents produced by microorganisms that kill or inhibit other microorganisms. This is the microbiologist's definition. A more broadened definition of an antibiotic includes any chemical of natural origin (from any type of cell) which has the effect to kill or inhibit the growth of other types cells. Since most clinically-useful antibiotics are produced by microorganisms and are used to kill or inhibit infectious Bacteria, we will follow the classic definition.

Antibiotics are low molecular-weight (non-protein) molecules produced as secondary metabolites, mainly by microorganisms that live in the soil. Most of these microorganisms form some type of a spore or other dormant cell, and there is thought to be some relationship (besides temporal) between antibiotic production and the processes of sporulation. Among the molds, the notable antibiotic producers are Penicillium and Cephalosporium , which are the main source of the beta-lactam antibiotics (penicillin and its relatives). In the Bacteria, the Actinomycetes, notably Streptomyces species, produce a variety of types of antibiotics including the aminoglycosides (e.g. streptomycin), macrolides (e.g. erythromycin), and the tetracyclines. Endospore-forming Bacillus species produce polypeptide antibiotics such as polymyxin and bacitracin. The table below (Table 4) is a summary of the classes of antibiotics and their properties including their biological sources.

Table 4. Classes of antibiotics and their properties

Chemical class	Examples	Biological	Spectrum	Mode of action

		source	(effective against)	
Beta-lactams (penicillins and cephalosporins)	Penicillin G, Cephalothin	Penicillium notatum and Cephalosporium species	Gram-positive bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Semisynthetic penicillin	Ampicillin, Amoxycillin		Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Clavulanic Acid	Clavamox is clavulanic acid plus amoxycillin	Streptomyces clavuligerus	Gram-positive and Gram-negative bacteria	Suicide inhibitor of beta-lactamases
Monobactams	Aztreonam	Chromobacter violaceum	Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Carboxypenems	Imipenem	Streptomyces cattleya	Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Aminoglycosides	Streptomycin	Streptomyces griseus	Gram-positive and Gram-negative bacteria	Inhibit translation (protein synthesis)
	Gentamicin	Micromonospora species	Gram-positive and Gram-negative bacteria esp. Pseudomonas	Inhibit translation (protein synthesis)
Glycopeptides	Vancomycin	Streptomyces orientales	Gram-positive bacteria, esp. Staphylococcus aureus	Inhibits steps in murein (peptidoglycan) biosynthesis and assembly

Lincomycins	Clindamycin	Streptomyces lincolnensis	Gram-positive and Gram-negative bacteria esp. anaerobic Bacteroides	Inhibits translation (protein synthesis)
Macrolides	Erythromycin	Streptomyces erythreus	Gram-positive bacteria, Gram-negative bacteria not enterics, Neisseria, Legionella, Mycoplasma	Inhibits translation (protein synthesis)
Polypeptides	Polymyxin	Bacillus polymyxa	Gram-negative bacteria	Damages cytoplasmic membranes
	Bacitracin	Bacillus subtilis	Gram-positive bacteria	Inhibits steps in murein (peptidoglycan) biosynthesis and assembly
Polyenes	Amphotericin	Streptomyces nodosus	Fungi	Inactivate membranes containing sterols
	Nystatin	Streptomyces noursei	Fungi (Candida)	Inactivate membranes containing sterols
Rifamycins	Rifampicin	Streptomyces mediterranei	Gram-positive and Gram-negative bacteria, Mycobacterium tuberculosis	Inhibits transcription (eubacterial RNA polymerase)
Tetracyclines	Tetracycline	Streptomyces species	Gram-positive and Gram-negative bacteria, Rickettsias	Inhibit translation (protein synthesis)
Semisynthetic tetracycline	Doxycycline		Gram-positive and Gram-negative bacteria, Rickettsias	Inhibit translation (protein synthesis)

			Ehrlichia, Borellia	
Chloramphenicol	Chloramphenicol	Streptomyces venezuelae	Gram-positive and Gram-negative bacteria	Inhibits translation (protein synthesis)

Antimicrobial Agents Used in the Treatment of Infectious Disease

The modern era of antimicrobial chemotherapy began in 1929 with Fleming's discovery of the powerful bactericidal substance penicillin, and Domagk's discovery in 1935 of synthetic chemicals (sulfonamides) with broad antimicrobial activity. In the early 1940's, spurred partially by the need for antibacterial agents in WW II, penicillin was isolated, purified and injected into experimental animals, where it was found to not only cure infections but also to possess incredibly low toxicity for the animals. This fact ushered into being the age of antibiotic chemotherapy and an intense search for similar antimicrobial agents of low toxicity to animals that might prove useful in the treatment of infectious disease. The rapid isolation of streptomycin, chloramphenicol and tetracycline soon followed, and by the 1950's, these and several other antibiotics were in clinical usage.

The most important property of a clinically-useful antimicrobial agent, especially from the patient's point of view, is its **selective toxicity**, i.e., that the agent acts in some way that inhibits or kills bacterial pathogens but has little or no toxic effect on the animal taking the drug This implies that the biochemical processes in the bacteria are in some way different from those in the animal cells, and that the advantage of this difference can be taken in chemotherapy. Antibiotics may have a cidal (killing) effect or a static (inhibitory) effect on a range of microbes. The range of bacteria or other microorganisms that are affected by a certain antibiotic are is expressed as its **spectrum of action**. Antibiotics effective against procaryotes which kill or inhibit a wide range of Gram-positive and Gram-negative bacteria are said to be **broad spectrum** . If effective mainly against Gram-positive or Gram-negative bacteria, they are **narrow spectrum** . If effective against a single organism or disease, they are referred to as **limited spectrum**.

Kinds of Antimicrobial Agents and their Primary Modes of Action

1. Cell wall synthesis inhibitors Cell wall synthesis inhibitors generally inhibit some step in the synthesis of bacterial peptidoglycan. Generally they exert their selective toxicity against eubacteria because human cells lack cell walls.

Beta lactam antibiotics Chemically, these antibiotics contain a 4-membered beta lactam ring. They are the products of two groups of fungi, Penicillium and Cephalosporium molds, and are correspondingly represented by the penicillins and cephalosporins. The beta lactam antibiotics inhibit the last step in peptidoglycan synthesis, the final cross-linking between between peptide side chains, mediated by bacterial carboxypeptidase and transpeptidase enzymes . Beta lactam antibiotics are normally bactericidal and require that cells be actively growing in order to exert their toxicity.

Natural penicillins, such as **Penicillin G** or **Penicillin V**, are produced by fermentation of Penicillium chrysogenum. They are effective against streptococcus, gonococcus and staphylococcus, except where resistance has developed. They are considered narrow spectrum since they are not effective against Gram-negative rods.

Semisynthetic penicillins first appeared in 1959. A mold produces the main part oif the molecule (6-aminopenicillanic acid) which can be modified chemically by the addition of side shains. Many of these compounds have been developed to have distinct benefits or advantages over penicillin G, such as increased spectrum of activity (effectiveness against Gram-negative rods), resistance to penicillinase, effectiveness when administered orally, etc. **Amoxycillin** and **Ampicillin** have broadened spectra against Gram-negatives and are effective orally; **Methicillin** is penicillinase-resistant.

Clavulanic acid is a chemical sometimes added to a semisynthetic penicillin preparation. Thus, **amoxycillin** plus **clavulanate is clavamox** or **augmentin**. The clavulanate is not an antimicrobial agent. It inhibits beta lactamase enzymes and has given extended life to penicillinase-sensitive beta lactams.

Although nontoxic, penicillins occasionally cause death when administered to persons who are allergic to them. In the U.S. there are 300 - 500 deaths annually due to penicillin allergy. In allergic individuals the beta lactam molecule attaches to a serum protein which initiates an IgE-mediated inflammatory response.

Cephalolsporins are beta lactam antibiotics with a similar mode of action to penicillins that are produced by species of Cephalosporium. The have a low toxicity and a somewhat broader spectrum than natural penicillins. They are often used as penicillin substitutes, against Gram-negative bacteria, and in surgical prophylaxis. They are subject to degradation by some bacterial beta-lactamases, but they tend to be resistant to beta-lactamases from S. aureus.

Bacitracin is a polypeptide antibiotic produced by *Bacillus* species. It prevents cell wall growth by inhibiting the release of the muropeptide subunits of peptidoglycan from the lipid carrier molecule that carries the subunit to the outside of the membrane Teichoic acid synthesis, which requires the same carrier, is also inhibited. Bacitracin has a high toxicity which precludes its systemic use. It is present in many topical antibiotic preparations, and since it is not absorbed by the gut, it is given to "sterilize" the bowel prior to surgery.

2. Cell membrane inhibitors disorganize the structure or inhibit the function of bacterial membranes. The integrity of the cytoplasmic and outer membranes is vital to bacteria, and compounds that disorganize the membranes rapidly kill the cells. However, due to the similarities in phospholipids in eubacterial and eukaryotic membranes, this action is rarely specific enough to permit these compounds to be used systemically. The only antibacterial antibiotic of clinical importance that acts by this mechanism is **Polymyxin**, produced by Bacillus polymyxis. Polymyxin is effective mainly against Gram-negative bacteria and is usually limited to topical usage. Polymyxins bind to membrane phospholipids and thereby interfere with membrane function. Polymyxin is occasionally given for urinary tract infections caused by Pseudomonas that are gentamicin, carbenicillin and tobramycin resistant. The balance between effectiveness and damage to the kidney and other organs is

dangerously close, and the drug should only be given under close supervision in the hospital.

3. Protein synthesis inhibitors Many therapeutically useful antibiotics owe their action to inhibition of some step in the complex process of translation. Their attack is always at one of the events occurring on the ribosome and rather than the stage of amino acid activation or attachment to a particular tRNA. Most have an affinity or specificity for 70S (as opposed to 80S) ribosomes, and they achieve their selective toxicity in this manner. The most important antibiotics with this mode of action are the **tetracyclines**, **chloramphenicol**, the **macrolides** (e.g. erythromycin) and the aminoglycosides (e.g. streptomycin).

The **aminoglycosides** are products of *Streptomyces* species and are represented by streptomycin, kanamycin, tobramycin and gentamicin. These antibiotics exert their activity by binding to bacterial ribosomes and preventing the initiation of protein synthesis. Aminoglycosides have been used against a wide variety of bacterial infections caused by Gram-positive and Gram-negative bacteria. **Streptomycin** has been used extensively as a primary drug in the treatment of tuberculosis. **Gentamicin** is active against many strains of Gram-positive and Gram-negative bacteria, including some strains of Pseudomonas aeruginosa. **Kanamycin** (a complex of three antibiotics, A, B and C) is active at low concentrations against many Gram-positive bacteria, including penicillin-resistant staphylococci. Gentamicin and **Tobramycin** are mainstays for treatment of Pseudomonas infections. An unfortunate side effect of aminoglycosides has tended to restrict their usage: prolonged use is known to impair kidney function and cause damage to the auditory nerves leading to deafness.

The **tetracyclines** consist of eight related antibiotics which are all natural products of *Streptomyces*, although some can now be produced semisynthetically. **Tetracycline**, **chlortetracycline** and **doxycycline** are the best known. The tetracyclines are broad-spectrum antibiotics with a wide range of activity against both Gram-positive and Gram-negative bacteria. The tetracyclines act by blocking the binding of aminoacyl tRNA to the A site on the ribosome. Tetracyclines inhibit protein

synthesis on isolated 70S or 80S (eukaryotic) ribosomes, and in both cases, their effect is on the small ribosomal subunit. However, most bacteria possess an active transport system for tetracycline that will allow intracellular accumulation of the antibiotic at concentrations 50 times as great as that in the medium. This greatly enhances its antibacterial effectiveness and accounts for its specificity of action, since an effective concentration cannot be accumulated in animal cells. Thus a blood level of tetracycline which is harmless to animal tissues can halt protein synthesis in invading bacteria.

The tetracyclines have a remarkably low toxicity and minimal side effects when taken by animals. The combination of their broad spectrum and low toxicity has led to their overuse and misuse by the medical community and the wide-spread development of resistance has reduced their effectiveness. Nonetheless, tetracyclines still have some important uses, such as in the treatment of Lyme disease.

Chloramphenicol has a broad spectrum of activity but it exerts a bacteriostatic effect. It is effective against intracellular parasites such as the rickettsiae. Unfortunately, aplastic anemia, which is dose related develops in a small proportion (1/50,000) of patients. Chloramphenicol was originally discovered and purified from the fermentation of a Streptomyces, but currently it is produced entirely by chemical synthesis. Chloramphenicol inhibits the bacterial enzyme peptidyl transferase thereby preventing the growth of the polypeptide chain during protein synthesis.

Chloramphenicol is entirely selective for 70S ribosomes and does not affect 80S ribosomes. Its unfortunate toxicity towards the small proportion of patients who receive it is in no way related to its effect on bacterial protein synthesis. However, since mitochondria probably originated from procaryotic cells and have 70S ribosomes, they are subject to inhibition by some of the protein synthesis inhibitors including chloroamphenicol. This likely explains the toxicity of chloramphenicol. The eukaryotic cells most likely to be inhibited by chloramphenicol are those undergoing rapid multiplication, thereby rapidly synthesizing mitochondria. Such cells include the blood forming cells of the bone marrow, the inhibition of which could present as aplastic anemia. Chloramphenicol was once a highly prescribed antibiotic and a

number of deaths from anemia occurred before its use was curtailed. Now it is seldom used in human medicine except in life-threatening situations (e.g. typhoid fever).

The **Macrolides** are a family of antibiotics whose structures contain large lactone rings linked through glycoside bonds with amino sugars. The most important members of the group are **erythromycin** and **oleandomycin**. Erythromycin is active against most Gram-positive bacteria, *Neisseria*, *Legionella* and *Haemophilus*, but not against the *Enterobacteriaceae*. Macrolides inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit. Binding inhibits elongation of the protein by peptidyl transferase or prevents translocation of the ribosome or both. Macrolides are bacteriostatic for most bacteria but are cidal for a few Gram-positive bacteria.

4. Effects on Nucleic Acids Some chemotherapeutic agents affect the synthesis of DNA or RNA, or can bind to DNA or RNA so that their messages cannot be read. Either case, of course, can block the growth of cells. The majority of these drugs are unselective, however, and affect animal cells and bacterial cells alike and therefore have no therapeutic application. Two nucleic acid synthesis inhibitors which have selective activity against procaryotes and some medical utility are nalidixic acid and rifamycins.

Nalidixic acid is a synthetic chemotherapeutic agent which has activity mainly against Gram-negative bacteria. Nalidixic acid belongs to a group of compounds called **quinolones**. Nalidixic acid is a bactericidal agent that binds to the DNA gyrase enzyme (topoisomerase) which is essential for DNA replication and allows supercoils to be relaxed and reformed. Binding of the drug inhibits DNA gyrase activity.

Some quinolones penetrate macrophages and neutrophils better than most antibiotics and are thus useful in treatment of infections caused by intracellular parasites. However, the main use of nalidixic acid is in treatment of lower urinary tract infections (UTI). The compound is unusual in that it is effective against several types of Gram-negative bacteria such as *E. coli, Enterobacter aerogenes, K. pneumoniae* and species which are common causes of UTI. It is not usually effective

against *Pseudomonas aeruginosa*, and Gram-positive bacteria are resistant. However, a fluoroquinolone, Ciprofloxacin (Cipro) was recently recommended as the drug of choice for prophylaxis and treatment of anthrax.

The **rifamycins** are also the products of *Streptomyces*. **Rifampicin** is a semisynthetic derivative of rifamycin that is active against Gram-positive bacteria (including *Mycobacterium tuberculosis*) and some Gram-negative bacteria. Rifampicin acts quite specifically on eubacterial RNA polymerase and is inactive towards RNA polymerase from animal cells or towards DNA polymerase. The antibiotic binds to the beta subunit of the polymerase and apparently blocks the entry of the first nucleotide which is necessary to activate the polymerase, thereby blocking mRNA synthesis. It has been found to have greater bactericidal effect against M .tuberculosis than other anti-tuberculosis drugs, and it has largely replaced isoniazid as one of the front-line drugs used to treat the disease, especially when isoniazid resistance is indicated. It is effective orally and penetrates well into the cerebrospinal fluid and is therefore useful for treatment of tuberculosis meningitis and meningitis caused by Neisseria meningitidis.

5. Competitive Inhibitors The competitive inhibitors are mostly all synthetic chemotherapeutic agents. Most are "growth factor analogs" which are structurally similar to a bacterial growth factor but which do not fulfill its metabolic function in the cell. Some are bacteriostatic and some are bactericidal.

Sulfonamides were introduced as chemotherapeutic agents by Domagk in 1935, who showed that one of these compounds (prontosil) had the effect of curing mice with infections caused by beta-hemolytic streptococci. Chemical modifications of the compound sulfanilamide gave compounds with even higher and broader antibacterial activity. The resulting sulfonamides have broadly similar antibacterial activity, but differ widely in their pharmacological actions. Bacteria which are almost always sensitive to the sulfonamides include *Streptococcus pneumoniae*, beta-hemolytic streptococci and *E. coli*. The sulfonamides have been extremely useful in the treatment of uncomplicated UTI caused by E. coli, and in the treatment of meningococcal meningitis (because they cross the blood-brain barrier).

The sulfonamides (e.g. **Gantrisin**) and **Trimethoprim** are inhibitors of the bacterial enzymes required for the synthesis of tetrahydofolic acid (THF), the vitamin form of folic acid essential for 1-carbon transfer reactions. Sulfonamides are structurally similar to para aminobenzoic acid (PABA), the substrate for the first enzyme in the THF pathway, and they competitively inhibit that step . Trimethoprim is structurally similar to dihydrofolate (DHF) and competitively inhibits the second step in THF synthesis mediated by the DHF reductase. Animal cells do not synthesize their own folic acid but obtain it in a preformed fashion as a vitamin. Since animals do not make folic acid, they are not affected by these drugs, which achieve their selective toxicity for bacteria on this basis.

Three additional synthetic chemotherapeutic agents have been used in the treatment of tuberculosis: **isoniazid (INH)**, **paraaminosalicylic acid (PAS)**, and **ethambutol**. The usual strategy in the treatment of tuberculosis has been to administer a single antibiotic (historically streptomycin, but now, most commonly, rifampicin is given) in conjunction with INH and ethambutol. Since the tubercle bacillus rapidly develops resistance to the antibiotic, ethambutol and INH are given to prevent outgrowth of a resistant strain. It must also be pointed out that the tubercle bacillus rapidly develops resistance to ethambutol and INH if either drug is used alone. Ethambutol inhibits incorporation of mycolic acids into the mycobacterial cell wall. Isoniazid has been reported to inhibit mycolic acid synthesis in mycobacteria and since it is an analog of pyridoxine (Vitamin B6) it may inhibit pyridoxine catalyzed reactions as well. Isoniazid is activated by a mycobacterial peroxidase enzyme and destroys several targets in the cell. PAS is an anti-folate. PAS was once a primary anti-tuberculosis drug, but now it is a secondary agent, having been largely replaced by ethambutol.

Bacterial resistance to antibiotics

Penicillin became generally available for treatment of bacterial infections, especially those caused by staphylococci and streptococci, about 1946. Initially, the antibiotic was effective against all sorts of infections caused by these two Gram-positive bacteria. Resistance to penicillin in some strains of staphylococci was recognized

almost immediately. (Resistance to penicillin today occurs in as many as 80% of all strains of *Staphylococcus aureus*). Surprisingly, *Streptococcus pyogenes* (Group A strep) have never fully developed resistance to penicillin and it remains a reasonable choice antibiotic for many types of streptococcal infections. Natural penicillins have never been effective against most Gram-negative pathogens (e.g. *Salmonella, Shigella, Bordetella pertussis, Yersinia pestis, Pseudomonas*) with the notable exception of *Neisseria gonorrhoeae*. Gram-negative bacteria are inherently resistant because their vulnerable cell wall is protected by an outer membrane that prevents permeation of the penicillin molecule.

The period of the late 1940s and early 1950s saw the discovery and introduction of streptomycin, chloramphenicol, and tetracycline, and the age of antibiotic chemotherapy came into full being. These antibiotics were effective against the full array of bacterial pathogens including Gram-positive and Gram-negative bacteria, intracellular parasites, and the tuberculosis bacillus. However, by 1953, during a *Shigella* outbreak in Japan, a strain of the dysentery bacillus was isolated which was multiple drug resistant, exhibiting resistance to chloramphenicol, tetracycline, streptomycin, and the sulfanilamides. There was also evidence mounting that bacteria could pass genes for multiple drug resistance between strains and even between species. It was also apparent that Mycobacterium tuberculosis was capable of rapid development of resistance to streptomycin which had become a mainstay in tuberculosis therapy.

By the 1960's it became apparent that some bacterial pathogens were developing resistance to antibiotic-after-antibiotic, at a rate faster than new antibiotics could be brought to market. A more conservative approach to the use of antibiotics has not been fully accepted by the medical and agricultural communities, and the problems of emerging multiple-drug resistant pathogens still loom. The most important pathogens to emerge in multiple drug resistant forms so far have been *Mycobacterium tuberculosis* and *Staphylococcus aureus*.

The basis of bacterial resistance to antibiotics

Inherent (Natural) Resistance Bacteria may be inherently resistant to an antibiotic. For example, a streptomycete has some gene that is responsible for resistance to its own antibiotic; or a Gram-negative bacterium has an outer membrane that establishes a permeability barrier against the antibiotic; or an organism lacks a transport system for the antibiotic; or it lacks the target or reaction that is hit by the antibiotic.

Acquired Resistance Bacteria can develop resistance to antibiotics, e.g. bacterial populations previously-sensitive to antibiotics become resistant. This type of resistance results from changes in the bacterial genome. Acquired resistance is driven by two genetic processes in bacteria: (1) mutation and selection (sometimes referred to as vertical evolution); (2) exchange of genes between strains and species (sometimes called horizontal evolution).

Vertical evolution is strictly a matter of Darwinian evolution driven by principles of natural selection: a spontaneous mutation in the bacterial chromosome imparts resistance to a member of the bacterial population. In the selective environment of the antibiotic, the wild type (non mutants) are killed and the resistant mutant is allowed to grow and flourish. The mutation rate formost bacterial genes is approximately 10^{-8}. This means that if a bacterial population doubles from 10^8 cells to 2×10^8 cells, there is likely to be a mutant present for any given gene. Since bacteria grow to reach population densities far in excess of 10^9 cells, such a mutant could develop from a single generation during 15 minutes of growth.

Horizontal evolution is the acquisition of genes for resistance from another organism. For example, a streptomycete has a gene for resistance to streptomycin (its own antibiotic), but somehow that gene escapes and gets into *E. coli* or *Shigella*. Or, more likely, Some bacterium develops genetic resistance through the process of mutation and selection and then donates these genesto some other bacterium through one of several processes for genetic exchange that exist in bacteria.

Bacteria are able to exchange genes in nature by three processes: conjugation, transduction and transformation. **Conjugation** involves cell-to-cell contact as DNA

crosses a sex pilus from donor to recipient. During **transduction**, a virus transfers the genes between mating bacteria. In **transformation**, DNA is acquired directly from the environment, having been released from another cell. Genetic recombination can follow the transfer of DNA from one cell to another leading to the emergence of a new genotype (recombinant). It is common for DNA to be transferred as plasmids between mating bacteria. Since bacteria usually develop their genes for drug resistance on plasmids (called resistance transfer factors, or RTFs), they are able to spread drug resistance to other strains and species during genetic exchange processes.

The combined effects of fast growth rates, high concentrations of cells, genetic processes of mutation and selection, and the ability to exchange genes, account for the extraordinary rates of adaptation and evolution that can be observed in the bacteria. For these reasons bacterial adaptation (resistance) to the antibiotic environment seems to take place very rapidly in evolutionary time: bacteria evolve fast!

The medical problem of bacterial drug resistance

Obviously, if a bacterial pathogen is able to develop or acquire resistance to an antibiotic, then that substance becomes useless in the treatment of infectious disease caused by that pathogen (unless the resistance can somehow be overcome with secondary measures). So as pathogens develop resistance, we must find new (different) antibiotics to fill the place of the old ones in treatment regimes. Hence, natural penicillins have become useless against staphylococci and must be replaced by other antibiotics; tetracycline, having been so widely used and misused for decades, has become worthless for many of the infections that once designated it as a "wonder drug".

Not only is there a problem in finding new antibiotics to fight old diseases (because resistant strains of bacteria have emerged), there is a parallel problem to find new antibiotics to fight new diseases. In the past two decades, many "new" bacterial diseases have been discovered (Legionnaire's disease, gastric ulcers, Lyme disease,

toxic shock syndrome, "skin-eating" streptococci). We are only now able to examine patterns of susceptibility and resistance to antibiotics among new pathogens that cause these diseases. Broad patterns of resistance exist in these pathogens, and it seems likely that we will soon need new antibiotics to replace the handful that are effective now against these bacteria, especially as resistance begins to emerge among them in the selective environment antibiotic chemotherapy.

IMPORTANT GROUPS OF PROCARYOTES

BACTERIA

Phylogenetic analysis of the **Bacteria** has demonstrated the existence of at least 13 distinct groups, but many groups consist of members that are phenotypically and physiologically unrelated, and sometimes phylogenetically unrelated. The current edition of Bergey's Manual of Systematic Bacteriology (2001) recognizes 23 distinct phyla of Bacteria (Phylum is the highest taxon in a Domain), but there may still be great variation in phenotype among members. Below we discuss the major groups of Bacteria based on morphology, physiology, or ecology, and often use informal, but familiar, terms to identify them.

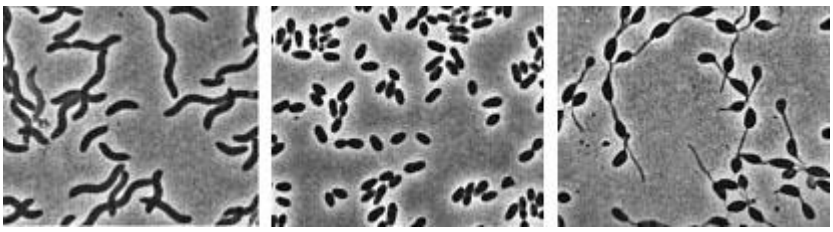
Photosynthetic purple and green bacteria. These bacteria conduct **anoxygenic photosynthesis**, also called **bacterial photosynthesis**. Bacterial photosynthesis differs from plant-type (oxygenic) photosynthesis in several ways. Bacterial photosynthesis does not produce O_2; in fact, it only occurs under anaerobic conditions. Bacterial photosynthesis utilizes a type of chlorophyll other than chlorophyll *a,* and only one photosystem, photosystem I. The electron donor for bacterial photosynthesis is never H_2O but may be H_2, H_2S or S^o, or certain organic compounds. The light-absorbing pigments of the purple and green bacteria consist of bacterial chlorophylls and carotenoids. Phycobilins, characteristic of the cyanobacteria, are not found. Many purple and green sulfur bacteria store elemental sulfur as a reserve material that can be further oxidized to SO_4 as a photosynthetic electron donor.

The **purple and green bacteria** may use H_2S during photosynthesis in the same manner that cyanobacteria or algae or plants use H_2O as an electron donor for autotrophic CO_2 reduction (the "dark reaction" of photosynthesis). Or they may utilize organic compounds as electron donors for photosynthesis. For example, *Rhodobacter* can use light as an energy source while oxidizing succinate or butyrate in order to obtain electrons for CO_2 fixation.

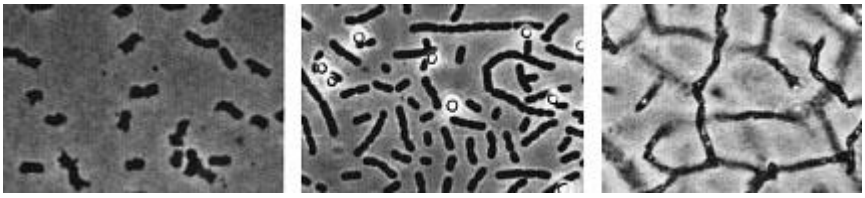
The bacterium that became an endosymbiont of eucaryotes and evolved into mitochondria is thought to be a relative of the purple nonsulfur bacteria. This conclusion is based on similar metabolic features of mitochondria and purple nonsulfur bacteria and on comparisons of the base sequences in their 16S rRNAs.

Figure 1. Photomicrographs (phase contrast and and ordinary illumination) of various photosynthetic bacteria. Magnifications are about 1400X. The purple and green bacteria exhibit a full range of procaryotic morphologies, as these photomicrographs illustrate. Diversity among their phylogenetic relationships is also noted.

A. Purple sulfur bacteria (L to R): *Chromatium vinosum, Thiospirillum jenense, Thiopedia rosea*.



B. Purple nonsulfur bacteria (L to R): *Rhodospirillum rubrum, Rhodobacter sphaeroides, Rhodomicrobium vannielii.* The purple nonsulfur bacteria are in the Alphaproteobacteria, which also includes *Rhizobium, Agrobacterium* and the Rickettsias. The latter bacteria represent a direct lineage to mitochondria.



C. Green sulfur bacteria (L to R): *Chlorobium limicola, Prosthecochloris aestuarii, Pelodictyon clathratiforme.* The Green sulfur bacteria represent a distinct phylogenetic lineage and cluster in their own phylum represented by *Chlorobium* .

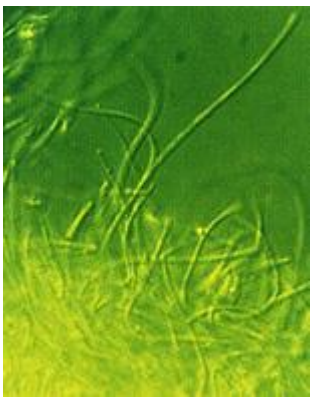


Figure 2. Green nonsulfur bacterium, *Chloroflexus* (T.D. Brock). *Chloroflexus* also represents a phylogenetically distinct group of green bacteria. *Chloroflexus* is a thermophilic, filamentous gliding bacterium.

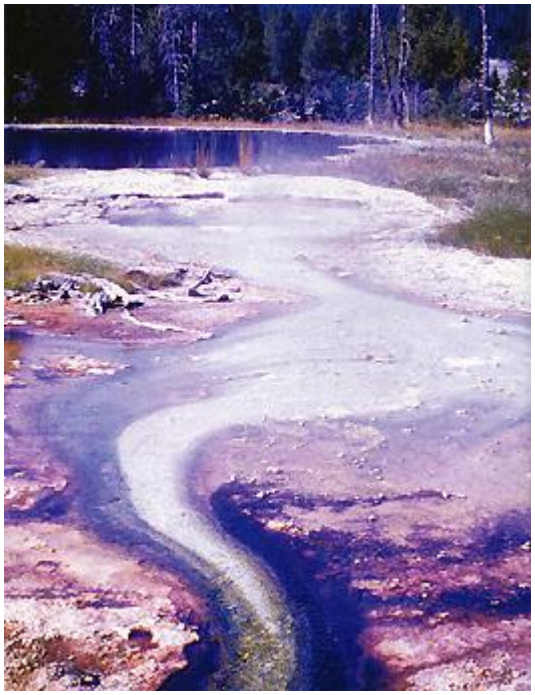


Figure 3. Photosynthetic procaryotes growing in a hot spring run-off channel (T.D. Brock). The white area of the channel is too hot for photosynthetic life, but as the water cools along a gradient, the colored phototrophic bacteria colonize and

ultimately construct the colored microbial mats composed of a consortium of photosynthetic microorganisms.

Cyanobacteria. The cyanobacteria deserve special emphasis because of their great ecological importance in the global carbon, oxygen and nitrogen cycles, as well as their evolutionary significance in relationship to plants. Photosynthetic cyanobacteria have chlorophyll *a* and carotenoids in addition to some unusual accessory pigments named **phycobilins**. The blue pigment, **phycocyanin** and the red one, **phycoerythrin**, absorb wavelengths of light for photosynthesis that are missed by chlorophyll and the carotenoids. Within the cytoplasm of cyanobacteria are numerous layers of membranes, often parallel to one another. These membranes are photosynthetic thylakoids that resemble those found in chloroplasts, which, in fact, correspond in size to the entire cyanobacterial cell. The main storage product of the cyanobacteria is glycogen, and glycogen inclusions may be seen in the cytoplasm of the cells. Cyanobacteria are thought to have given rise to eucaryotic chloroplasts during the evolutionary events of endosymbiosis. In biochemical detail, cyanobacteria are especially similar to the chloroplasts of red algae (*Rhodophyta*).

Most cyanobacteria have a mucilaginous sheath, or coating, which is often deeply pigmented, particularly in species that occur in terrestrial habitats. The colors of the sheaths in different species include light gold, yellow, brown, red, green, blue, violet, and blue-black. It is these pigments that impart color to individual cells and colonies as well as to "blooms" of cyanobacteria in aquatic environments

Figure 4. Some common cyanobacteria L to R: *Oscillatoria,* a filamentous species common in fresh water and hot springs; *Nostoc,* a sheathed communal species; *Anabaena,* a nitrogen-fixing species. The small cell with an opaque surface (third from right) in the anabaena filament is a heterocyst, a specialized cell for nitrogen fixation. The large bright

cell in the filament is a type of spore called an akinete; *Synechococcus,* a unicelluar species in marine habitats and hot springs. *Synechococcus* is among the most important photosynthetic bacteria in the marine environment, estimated to account for about 25 percent of the primary production that occurs in typical marine habitats.

Although thousands of cyanobacteria have been observed, only about 200 species have been identified as distinct, free-living, nonsymbiotic procaryotes. Relative to other oxygenic phototrophs, cyanobacteria often grow under fairly extreme environmental conditions such as high temperature and salinity . They are the only oxygenic phototrophs present in many hot springs of the Yellowstone ecosystem; and in frigid lakes and oceans of Antarctica, they form luxuriant mats 2 to 4 centimeters thick in water beneath more than five meters of permanent ice. However, cyanobacteria are absent in acidic waters where their eucaryotic counterparts, the algae, may be abundant.

Layered chalk deposits called **stromatolites**, which exhibit a continuous geologic record covering 2.7 billion years, are produced when colonies of cyanobacteria bind calcium-rich sediments. Today, stromatolites are formed in only a few places, such as shallow pools in hot dry climates. The abundance of cyanobacteria in the fossil record is evidence of the early development of the cyanobacteria and their important role in elevating the level of free oxygen in the atmosphere of the early Earth.

Cyanobacteria often form filaments and may grow in large masses or "tufts" one meter or more in length. Some are unicellular, a few form branched filaments, and a few form irregular plates or irregular colonies. Cyanobacterial cells usually divide by binary fission, and the resulting progeny cells may separate to form new colonies. In addition, filaments may break into fragments, called **hormogonia**, which separate and develop into new colonies. As in other filamentous or colonial bacteria, the cells of cyanobacteria may joined by their walls or by mucilaginous sheaths, but each cell is an independent unit of life.

As true Bacteria, cyanobacteria contain peptidoglycan or murein in their cell walls. Most cyanobacteria have a Gram-negative type cell wall that consists of an outer membrane component, even though they may show a distant phylogenetic

relationship with certain Gram-positive bacteria. Some of the filamentous cyanobacteria are motile by means gliding or rotating around a longitudinal axis. Short segments (hormogonia) may break off from a cyanobacterial colony and glide away from their parent colony at rates as rapid as 10 micrometers per second. The mechanism for this movement is unexplained but may be connected to the extrusion of slime (mucilage) through small pores in their cell wall, together with contractile waves in one of the surface layers of the wall.

Cyanobacteria are found in most aerobic environments where water and light are available for growth. Mainly they live in fresh water and marine habitats. Those inhabiting the surface layers of water are part of a complex microbial community called **plankton**. Planktonic cyanobacteria usually contain cytoplasmic inclusions called **gas vesicles** which are hollow protein structures filled with various gases. The vesicles can be inflated or deflated with gases allowing the organisms to maintain buoyancy and to float at certain levels in the water. Thus, the cyanobacteria can regulate their position in the water column to meet their optimal needs for photosynthesis, oxygen, and light-shielding. When numerous cyanobacteria become unable to regulate their gas vesicles properly (for example, because of extreme fluctuations of temperature or oxygen supply), they may float to the surface of a body of water and form visible "blooms". A planktonic species related to *Oscillatoria* gives rise to the redness (and the name) of the Red Sea.

The cyanobacteria have very few harmful effects on plants or animals. They may be a nuisance if they bloom in large numbers and then die and decay in bodies of fresh water that are used for drinking or recreational purposes. Many cyanobacteria are responsible for the earthy odors and flavors of fresh waters, including drinking waters, due to the production of compounds called **geosmins.** Some cyanobacteria that form blooms secrete poisonous substances that are toxic for animals that ingest large amounts of the contaminated water.

Many marine cyanobacteria occur in limestone (calcium carbonate) or lime-rich substrates, such as coral algae and the shells of mullosks. Some fresh water species,

particularly those that grow in hot springs, often deposit thick layers of lime in their colonies.

Some cyanobacteria can fix nitrogen. In filamentous cyanobacteria, nitrogen fixation often occurs in **heterocysts**, which are specialized, enlarged cells, usually distributed along the length of a filament or at the end of a filament. Heterocysts have intercellular connections to adjacent vegetative cells, and there is continuous movement of the products of nitrogen fixation moving from heterocysts to vegetative cells, and the products of photosynthesis moving from vegetative cells to heterocysts. Heterocysts are low in phycobilin pigments and have only photosystem I. They lack the oxygen-evolving photosystem II. Furthermore, they are surrounded in a thickened, specialized glycolipid cell wall that slows the rate of diffusion of O_2 into the cell. Any O_2 that diffuses into the heterocyst is rapidly reduced by hydrogen, a byproduct of N_2 fixation, or is expelled through the wall of the heterocyst. The process of nitrogen fixation, specifically the enzyme nitrogenase, only functions in anaerobic conditions so the organism must maintain these oxygen-free compartments in order for N_2fixation to occur.

In addition to the heterocysts, some cyanobacteria form resistant spores called **akinetes** enlarged cells around which thickened outer walls develop. Akinetes are resistant to heat, freezing and drought (desiccation) and thus allow the cyanobacteria to survive unfavorable environmental conditions. They are functionally analogous to bacterial endospores, but they bear little resemblance and lack the extraordinary resistance properties of endospores.

A few cyanobacteria are symbionts of liverworts, ferns, cycads, flagellated protozoa, and algae, sometimes occurring as endosymbionts of the eucaryotic cells. In the case of the water fern, *Azolla,* the cyanobacterial endophyte (a species of *Anabaena)* fixes nitrogen that becomes available to the plant. In addition, it is often the case that the photosynthetic partners of **lichens** are cyanobacteria.

The planktonic cyanobacteria fix an enormous amount of CO_2 during photosynthesis, and as "primary producers" they are the basis of the food chain in marine

environments. Their type of photosynthesis, which utilizes photosystem II, generates a substantial amount of oxygen present in the earth's atmosphere. Since many cyanobacteria can fix N_2 under certain conditions, they are one of the most significant free-living nitrogen-fixing procaryotes. Cyanobacteria carried out plant-type (oxygenic) photosynthesis for at least a billion and a half years before the emergence of plants, and cyanobacteria are believed to be the evolutionary forerunners of modern-day plant and algal chloroplasts. A group of phototrophic procaryotes, called **prochlorophytes** contain chlorophyll *a* and *b* but do **not** contain phycobilins. Prochlorophytes, therefore, resemble both cyanobacteria (because they are procaryotic and contain chlorophyll *a*) and the plant chloroplast (because they contain chlorophyll *b* instead of phycobilins). *Prochloron,*the first prochlorophyte discovered, is phenotypically very similar to certain plant chloroplasts and is the leading candidate for the type of bacterium that might have undergone endosymbiotic events that led to the development of the plant chloroplast.

Spirochetes are a phylogenetically distinct group of Bacteria which have a unique cell morphology and mode of motility. Spirochetes are very thin, flexible, spiral-shaped procaryotes that move by means of structures called **axial filaments** or **endoflagella**. The flagellar filaments are contained within a sheath between the cell wall peptidoglycan and an outer membrane. The filaments flex or rotate within their sheath which causes the cells to bend, flex and rotate during movement. Most spirochetes are free living (in muds and sediments), or live in associations with animals (e.g. in the oral cavity or GI tract). A few are pathogens of animals (e.g. leptospirosis in dogs, Syphilis in humans and Lyme disease in dogs and humans).

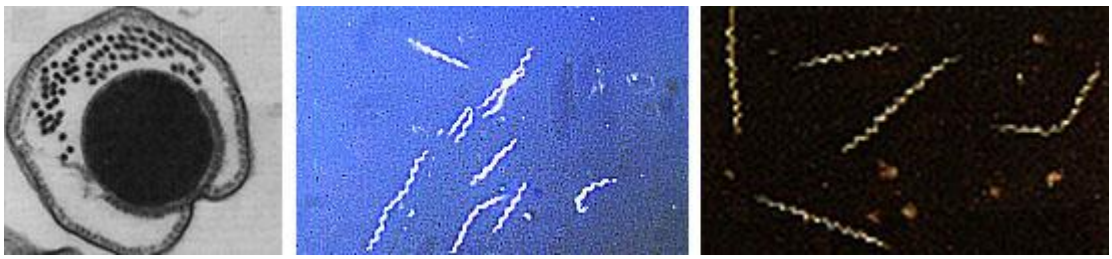


Figure 5. Spirochetes: A. Cross section of a spirochete showing the location of endoflagella between the inner membrane and outer sheath; B. *Borrelia burgdorferi,* the agent of Lyme disease; C. *Treponema pallidum,* the spirochete that causes syphilis.

Other Spiral-Shape and and Curved Bacteria. The main thing that unifies this group of bacteria is their spiral or vibrioid (curved) shape, although they are all classified among the Proteobacteria. Nontheless, in certain environments, their characteristic shape can instantly inform an observer of their identity. Bacteria referred to as "**spirilla**" are Gram-negative aerobic heterotrophic bacteria with a helical or spiral shape. Their metabolism is usually respiratory and never fermentative. Unlike spirochetes, they have a rigid cell wall and are motile by means of ordinary polar flagella. Spirilla are inhabitants of microaerophilic aquatic environments. Most spirilla require or prefer that oxygen in their environment be present in an amount that is well below atmospheric concentration. The *Rhodospirillaceae*are found in the Alpha group of Proteobacteria; *Spirillaceae* and *Oceanospirillaceae* are Gammaproteobacteria.

As inhabitants of marine and fresh waters many spirilla are endowed with some interesting properties. *Magnetospirillum*contains **magnetosomes** and exhibits the property of **magnetotaxis** (movement in relationship to the magnetic field of the earth). *Oceanospirillum* lives in marine habitats and is able to grow at NaCl concentrations as high as 9 percent. *Azospirillum* is a nitrogen-fixing bacterium that enters into a mutualistic symbiosis with certain tropical grasses and grain crops. Spirilla are thought to play a significant role in recycling of organic matter, particularly in aquatic environments.

Two pathogens of humans are found among the spiril forms in the Epsilon group of Proteobacteria. *Campylobacter jejuni* is an important cause of bacterial diarrhea, especially in children. The bacterium is transmitted via contaminated food, usually undercooked poultry or shellfish, or untreated drinking water. *Helicobacter pylori* is able to colonize the gastric mucosal cells of humans, i.e., the lining of the stomach, and it has been well established as the cause of peptic ulcers.

Bacteria with a curved rod or comma shape are referred to as "**vibrios**". Like the spiral forms, vibrios are very common bacteria in aquatic environments. They are found among the Gammaproteobacteria and have structural and metabolic properties that overlap with both the enterics and the pseudomonads. In Bergey's Manual

(2001) *Vibrionaceae* is a family on the level with *Enterobacteriaceae*. Vibrios are facultative like enterics, but they have polar flagella, are oxidase-positive, and dissimilate sugars in the same manner as the pseudomonads. In aquatic habitats they overlap with the *Pseudomonadaceae* in their ecology, although *Pseudomonas* species favor fresh water and vibrios prefer salt water. The genus *Vibrio* contains an important pathogen of humans, *Vibrio cholerae,* the cause of **Asiatic cholera**. Cholera is an intestinal disease with a pathology related to diarrheal diseases caused by the enteric bacteria.

Five species of marine vibrios exhibit the property of **bioluminescence**, the ability to emit light of a blue-green color. These bacteria may be found as saprophytes of dead fish or as symbionts of living fish and invertebrates in marine environments. Some grow in special organs of the fish and emit light for the benefit of the fish (to attract prey, or as a mating signal) in return for a protected habitat and supply of nutrients. The reaction leading to light emission, catalyzed by the enzyme **luciferase**, has been found to be the same in all procaryotes, and differs from light emission by eucaryotes such as the fire fly. Luciferase diverts electrons from the normal respiratory electron transport chain and causes formation of an excited peroxide that leads to emission of light.

The small vibrioid bacterium, *Bdellovibrio,*is a tiny curved rod that is a parasite of other Gram-negative bacteria, including *E. coli*. It preys on other bacteria by entering into the periplasmic space and obtaining nutrients from the cytoplasm of its host cell while undergoing an odd type of reproductive cycle. *Bdellovibrio*is a member of the Deltaproteobacteria.

The **Myxobacteria** are a group of **fruiting gliding bacteria** that comprise a unique order of Deltaproteobacteria. They exhibit a unique type of gliding motility. The vegetative cells move (glide) about together as a swarm, and then they aggregate together to form a multicellular fruiting body in which development and spore formation takes place. They exhibit the most complex behavioral patterns and life cycles of all known procaryotes. Myxobacteria are inhabitants of the soil. They

have a eucaryotic counterpart in nature in the *Myxomycetes*, or slime molds, and the two types of organisms are an example of parallel or **convergent evolution**, having adopted similar life styles in the soil environment.

The vegetative cells of myxobacteria are typical Gram-negative rods that glide across a substrate such as a decaying leaf or piece of animal dung, or colonies of other bacteria. They obtain nutrients from the substrate as they glide across it and they secrete a slime track which other myxobacterial cells preferentially follow. If their nutrients become exhausted, the cells signal to one another to aggregate and form a swarm of myxobacteria which eventually differentiate into a multicellular **fruiting body** that contains **myxospores**, a type of dormant cell descended from a differentiated vegetative cell. In the case of *Stigmatella*, the myxospores are packed into secondary structures called **cysts**, which develop at the tips of the fruiting body (Figure 6). The bright-colored fruiting bodies of myxobacteria, containing millions of cells and spores, can often be seen with the unaided eye on dung pellets and decaying vegetation in the soil.

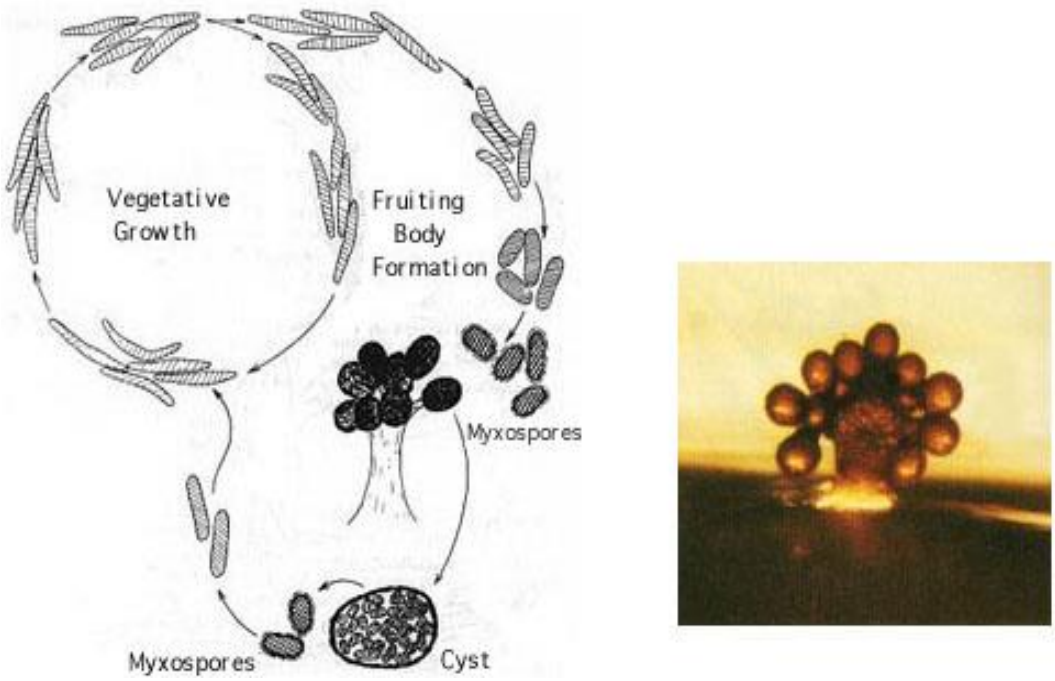


Figure 6. *Stigmatella aurantiaca*, a fruiting myxobacterium: L. Life Cycle R. Fruiting Body.

Lithotrophs. Lithotrophy, a type of metabolism that requires inorganic compounds as sources of energy. This metabolism is firmly established in both the Archaea and the Bacteria. The methanogens utilize H_2 as an energy source, and many extreme thermophiles use H_2S or elemental sulfur as a source of energy for growth. Lithotrophic Bacteria are typically Gram-negative species that utilize inorganic substrates including H_2, NH_3, NO_2, H_2S, S, Fe^{++}, and CO. Ecologically, the most important lithotrophic Bacteria are the **nitrifying bacteria**, *Nitrosomonas* and *Nitrobacter* that together convert NH_3 to NO_2, and NO_2 to NO_3, and the **colorless sulfur bacteria,** such as *Thiobacillus,* that oxidize H_2S to S and S to SO_4. Most lithotrophic bacteria are autotrophs, and in some cases, they may play an important role in primary production of organic material in nature. Lithotrophic metabolism does not extend to eucaryotes (unless a nucleated cell harbors lithotrophic endosymbiotic bacteria), and these bacteria are important in the biogeochemical cycles of the elements.

Figure 7. Lithotroph Habitats. A. Stream in Northern Wisconsin near Hayward is a good source of iron bacteria (John Lindquist). B. Bacteriologist J.C. Ensign of the University of Wisconsin observing growth of iron bacteria in a run-off channel from the Chocolate Pots along the Gibbon River, in Yellowstone National Park (K.Todar). C. An acid hot spring at the Norris Geyser Basin in Yellowstone is rich in iron and sulfur (T.D. Brock). D. A black smoker chimney in the deep sea emits iron sulfides at very high temperatures (270 to 380 degrees C).

Pseudomonads. "Pseudomonad" is an informal term for bacteria which morphologically and physiologically resemble members of the genus *Pseudomonas,* a

very diverse group of Gram-negative rods with a strictly-respiratory mode of metabolism. The term is usually applied to bacteria in the genera *Pseudomonas,* and *Xanthomonas,* which are Alphaproteobacteria, and to plant and animal pathogens such as *Burkholderia, Ralstonia* and *Acidovorax,* which are Betaproteobacteria. But many other related bacteria share their definitive characterictics, i.e., Gram-negative aerobic rods. The morphology and habitat of many pseudomonads sufficiently overlaps with the enterics (below) that microbiologists must quickly learn how to differentiate these two types of Gram-negative motile rods. Pseudomonads move by polar flagella; enterics such as *E. coli* swim by means of peritrichous flagella. Enterics ferment sugars such as glucose; pseudomonads generally do not ferment sugars. And most pseudomonads have an unusual cytochrome in their respiratory electron transport chain that can be detected in colonies by a colorimetric test called the **oxidase test**. Pseudomonads are typically oxidase- positive.

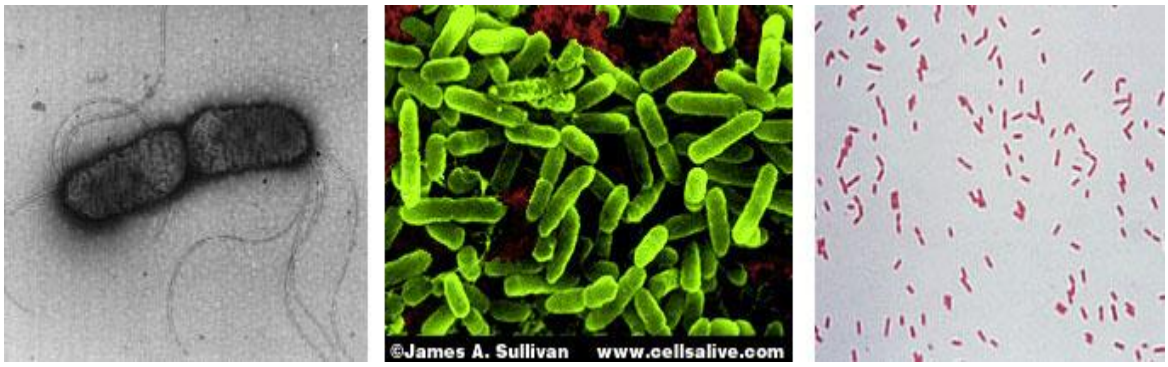


Figure 8. Profile of a pseudomonad: Gram-negative rods motile by polar flagella. A. Electron micrograph, negative stain. B. Scanning electron micrograph. C. Gram stain.

Most pseudomonads are free-living organisms in soil and water; they play an important role in decomposition, biodegradation, and the C and N cycles. The phrase "no naturally-occurring organic compound cannot be degraded by some microorganism" must have been coined to apply to members of the genus *Pseudomonas,* known for their ability to degrade hundreds of different organic compounds including insecticides, pesticides, herbicides, plastics, petroleum substances, hydrocarbons and other of the most refractory molecules in nature. However, they are usually unable to degrade biopolymers in their environment, such as cellulose and lignen, and their role in anaerobic decomposition is minimal.

There are about 150 species of *Pseudomonas*, but, especially among the plant pathogens, there are many strains and biovars among the species. These bacteria are frequently found as part of the normal flora of plants, but they are one of the most important bacterial pathogens of plants, as well. *Pseudomonas syringae* and *Xanthomonas* species cause a wide variety of plant diseases as discussed below. One strain of *Pseudomonas* that lives on the surfaces of plants can act as an "ice nucleus" which causes ice formation and inflicts frost damage on plants at one or two degrees *above* the conventional freezing temperature of water (0 degrees C). One *Pseudomonas* species is an important pathogen of humans, *Pseudomonas aeruginosa,* the quintessential opportunistic pathogen, which is a leading cause of hospital-acquired infections.

Among some interesting or important ecologic relatives of the pseudomonads are *Rhizobium*and *Bradyrhizobium,* species that fix nitrogen in association with leguminous plants, and related *Agrobacterium* species that cause tumors ("galls") in plants. These bacteria are discussed later in this article because of their special relationships with plants. Relatives of the pseudomonads also include the **methanotrophs** that can oxidize methane and other one-carbon compounds, the **azotobacters**, which are very prevalent free-living (nonsymbiotic) nitrogen-fixing bacteria.

Enterics. Enteric bacteria are Gram-negative rods with facultative anaerobic metabolism that live in the intestinal tracts of animals. This group consists of *Escherichia coli* and its relatives, the members of the family *Enterobacteriaceae.* Enteric bacteria are related phenotypically to several other genera of bacteria such as *Pseudomonas* and *Alcaligenes,* but are physiologically quite unrelated. Generally, a distinction can be made on the ability to ferment glucose: enteric bacteria all ferment glucose to acid end products while similar Gram-negative bacteria cannot ferment glucose. Because they are consistent members of the normal flora of humans, and because of their medical importance, an extremely large number of enteric bacteria have been isolated and characterized.

Escherichia coli is, of course, the type species of the enterics. *E. coli* is such a regular inhabitant of the intestine of humans that it is used by public health authorities as an indicator of fecal pollution of drinking water supplies, swimming beaches, foods, etc. *E. coli* is the most studied of all organisms in biology because of its occurrence, and the ease and speed of growing the bacteria in the laboratory. It has been used in hundreds of thousands of experiments in cell biology, physiology, and genetics, and was among the first cells for which the entire chromosomal DNA base sequence was determined. In spite of the knowledge gained about the molecular biology and physiology of *E. coli,* surprisingly little is known about its ecology, for example why it consistently associates with humans, how it helps its host, how it harms its host, etc. A few strains of *E. coli* are pathogenic (one is notorious, strain 0157:H7, that keeps turning up in raw hamburger headed for a fast-food restaurants). Pathogenic strains of *E. coli* cause **intestinal tract infections** (usually acute and uncomplicated, except in the very young), uncomplicated **urinary tract infections** and **neonatal meningitis.**

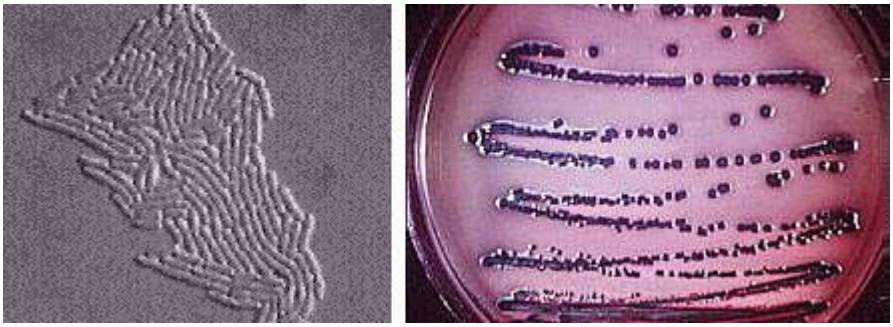


Figure 9. Left: *Escherichia coli* cells. Right: *E. coli* colonies on EMB Agar.

The enteric group also includes some other intestinal pathogens of humans such as *Shigella dysenteriae*, cause of **bacillary dysentery**, and *Salmonella typhimurium*, cause of **gastroenteritis**. *Salmonella typhi*, which infects via the intestinal route, causes **typhoid feve**r. Some bacteria that don't have an intestinal habitat resemble *E. coli* in enough ways to warrant inclusion in the enteric group. This includes *Proteus,* a common saprophyte of decaying organic matter, *Yersinia pestis,* which causes **bubonic plague**, and *Erwinia,* an important pathogen of plants.

Gram-negative pathogens. The Gram negative bacteria that are important pathogens of humans are found sattered throughout the Proteobacteria. In the Alphaproteobacteria, one finds the Rickettsias, a group of obligate intracellular parasites which are the cause of **typhus** and **Rocky Mountain Spotted fever**. In the Beta group, the agents of **whooping cough (pertussis)** (*Bordetella pertussis),* gonorrhea (*Neisseria gonorrhoeae*), and meningococcal meningitis (*Neisseria meningitidis*) are found. Among the Gamma group, *Pseudomonas aeruginosa,* the enterics, and *Vibrio cholerae* have already been mentioned. Likewise, the agents of Legionaires' pneumonia (*Legionella pneumophilia),* and childhood meningitis *(Haemophilus influenzae)* are Gammaproteobacteria. *Campylobacter* and *Helicobacter* are Epsilonproteobacteria. Most of these bacteria are discussed elsewhere in this article and/or in separate chapters which deal with their pathogenicity for humans.

Nitrogen-fixing organisms. This is a diverse group of procaryotes, reaching into phylogenetically distinct groups of Archaea and Bacteria. Members are unified only on the basis of their metabolic ability to "fix" nitrogen. **Nitrogen fixation** is the reduction of N_2 (atmospheric nitrogen) to NH_3 (ammonia). It is a complicated enzymatic process mediated by the enzyme **nitrogenase**. Nitrogenase is found only in procaryotes and is second only to RUBP carboxylase (the enzyme responsible for CO_2 fixation) as the most abundant enzyme on Earth.

The conversion of nitrogen gas (which constitutes about 80 percent of the atmosphere) to ammonia introduces nitrogen into the biological nitrogen cycle. Living cells obtain their nitrogen in many forms, but usually from ammonia (NH_3) or nitrates (NO_3), and never from N_2. Nitrogenase extracts N_2 from the atmosphere and reduces it to NH_3 in a reaction that requires substantial reducing power (electrons) and energy (ATP). The NH_3 is immediately assimilated into amino acids and proteins by subsequent cellular reactions. Thus, nitrogen from the atmosphere is fixed into living (organic) material.

Although a widespread trait in procaryotes, nitrogen fixation occurs in only a few select genera. Outstanding among them are the symbiotic bacteria *Rhizobium* and

Bradyrhizobium which form nodules on the roots of legumes. In this symbiosis the bacterium invades the root of the plant and fixes nitrogen which it shares with the plant. The plant provides a favorable habitat for the bacterium and supplies it with nutrients and energy for efficient nitrogen fixation. *Rhizobium* and *Bradyrhizobium* are Gram-negative aerobes related to the pseudomonads (above). An unrelated bacterium, an actinomycete (below), enters into a similar type of symbiosis with plants. The actinomycete, *Frankia,* forms nodules on the roots of several types of trees and shrubs, including alders (*Alnus*), wax myrtles (*Myrica*) and mountain lilacs (*Ceanothus*). They, too, fix nitrogen which is provided to their host in a useful form. This fact allows alder species to be "pioneer plants" (among the first to colonize) in newly-forming nitrogen-deficient soils. Still other bacteria live in regular symbiotic associations with plants on roots or leaves and fix nitrogen for their hosts, but they do not cause tissue hyperplasia or the formation of nodules.

Cyanobacteria are likewise very important in nitrogen fixation. Cyanobacteria provide fixed nitrogen, in addition to fixed carbon, for their symbiotic partners which make up lichens. This enhances the capacity for lichens to colonize bare areas where fixed nitrogen is in short supply. In some parts of Asia, rice can be grown in the same paddies continuously without the addition of fertilizers because of the presence of nitrogen fixing cyanobacteria. The cyanobacteria, especially *Anabaena*, occur in association with the small floating water fern *Azolla,* which forms masses on the paddies. Because of the nearly obligate association of *Azolla* with *Anabaena,* paddies covered with *Azolla* remain rich in fixed nitrogen.

In addition to symbiotic nitrogen-fixing bacteria, there are various free-living nitrogen-fixing procaryotes in both soil and aquatic habitats. Cyanobacteria may be able to fix nitrogen in virtually all habitats that they occupy. Clostridia and some methanogens fix nitrogen in anaerobic soils and sediments, including thermophilic environments. A common soil bacterium, *Azotobacter* is a vigorous nitrogen fixer, as is *Rhodospirillum*, a purple sulfur bacterium. Even *Klebsiella*, an enteric bacterium closely related to *E. coli,* fixes nitrogen. There is great scientific interest, of course, in knowing how one might move the genes for nitrogen fixation from a procaryote into a eucaryote such as corn or some other crop plant. The genetically engineered plant

might lose its growth requirement for costly ammonium or nitrate fertilizers and grow in nitrogen deficient soils.

Besides nitrogen fixation, bacteria play other essential roles in the processes of the nitrogen cycle. For example, saprophytic bacteria, decompose proteins releasing NH_3 in the process of **ammoniafication**. NH_3 is oxidized by lithotrophic *Nitrosomonas* species to NO_2 which is subsequently oxidized by *Nitrobacter* to NO_3. The overall conversion of NH_3 to NO_3 is called **nitrification**. NO_3 can be assimilated by cells as a source of nitrogen (**assimilatory nitrate reduction**), or certain bacteria can reduce NO_3 during a process called **anaerobic respiration**, wherein nitrate is used in place of oxygen as a terminal electron acceptor for a process analogous to aerobic respiration. In the case of anaerobic respiration, NO_3 is first reduced to NO_2, which is subsequently reduced to N_2O or N_2 or NH_3 (all gases). This process is called **denitrification** and it occurs in anaerobic environments where nitrates are present. If denitrification occurs in crop soils it may not be beneficial to agriculture if it converts utilizable forms of nitrogen (as in nitrate fertilizers) to nitrogen gases that will be lost into the atmosphere. One rationale for tilling the soil is to keep it aerobic in order to discourage denitrification processes in *Pseudomonas* and *Bacillus* which are ubiquitous inhabitants.

The **pyogenic cocci** are spherical bacteria which cause various suppurative (pus-producing) infections in animals. Included are the Gram-positive cocci *Staphylococcus aureus, Streptococcus pyogenes* and *Streptococcus pneumoniae,* and the Gram-negative cocci, *Neisseria gonorrhoeae* and *N. meningitidis.* These bacteria are leading pathogens of humans. It is estimated that they produce at least a third of all the bacterial infections of humans, including strep throat, pneumonia, food poisoning, various skin diseases and severe types of septic shock, gonorrhea and meningitis. *Staphylococcus aureus* is arguably the most successful of all bacterial pathogens because it has a very wide range of virulence determinants (so it can produce a wide range of infections) and it often occurs as normal flora of humans (on skin, nasal membranes and the GI tract), which ensures that it is readily transmitted from one individual to another. In terms of their phylogeny, physiology

and genetics, these genera of bacteria are quite unrelated to one another. They share a common ecology, however, as parasites of humans.

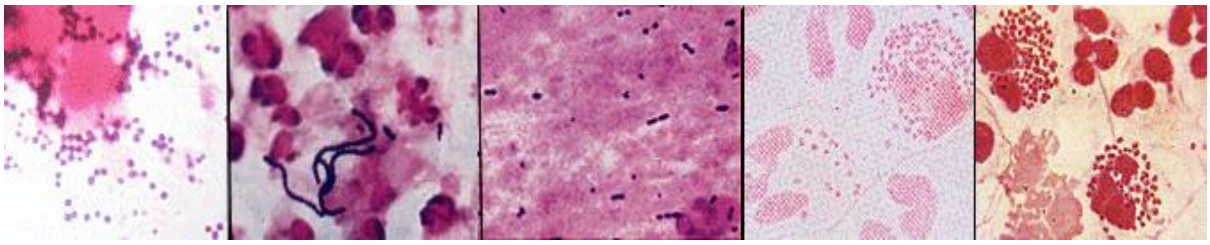


Figure 10. Gallery of pyogenic cocci, Gram stains of clinical specimens (pus), L to R: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*. The large cells with lobed nuclei are neutrophils. Pus is the outcome of the battle between phagocytes (neutrophils) and the invading cocci. As the bacteria are ingested and killed by the neutrophils, the neutrophils eventually lyse (rupture) and release their own components, plus the digested products of bacterial cells, which are the make-up of pus. As a defense against phagocytes the staphylococci and streptococci produce toxins that kill the neutrophils before they are able to ingest the bacteria. This contributes to the pus, and therefore these bacteria are "pyogenic" during their pathogenic invasions.

Two species of Staphylococcus live in association with humans: *Staphylococcus epidermidis* which lives normally on the skin and mucous membranes, and *Staphylococcus aureus* which may occur normally at various locales, but in particular on the nasal membranes (nares). *S. epidermidis* is rarely a pathogen and probably benefits its host by producing acids on the skin that retard the growth of dermatophytic fungi. *Staphylococcus aureus* always has the potential to cause disease and so is considered a pathogen. Different strains of *S. aureus* differ in the range of diseases they can cause, including boils and pimples, **wound infections**, **pneumonia, osteomyelitis**, **septicemia**, **food intoxication**, and **toxic shock syndrome**. *S. aureus* is the leading cause of **nosocomial (hospital-acquired) infections** by Gram-positive bacteria. Also, it is notoriously resistant to penicillin and many other antibiotics. Recently, a strain of *S. aureus* has been reported that is resistant to **EVERY** known antibiotic in clinical usage, which is a grim reminder that the clock is ticking on the lifetime of the usefulness of current antibiotics in treatment of infectious disease.

Streptococcus pypgenes, more specifically the **Beta-hemolytic Group A Streptococci**, like *S. aureus*, causes an array of suppurative diseases and toxinoses (diseases due to the production of a bacterial toxin), in addition to some autoimmune or allergic diseases. *S. pyogenes* is rarely found as normal flora (<1%), but it is the main streptococcal pathogen for man, most often causing tonsillitis or **strep throat**. Streptococci also invade the skin to cause localized infections and lesions, and produce toxins that cause **scarlet fever** and toxic shock. Sometimes, as a result of an acute streptococcal infection, anomalous immune responses are started that lead to diseases like **rheumatic fever** and **glomerulonephritis**, which are called **post-streptococcal sequelae**. Unlike the staphylococci, the streptococci have not developed widespread resistance to penicillin and the other beta lactam antibiotics, so that the beta lactams remain drugs of choice for the treatment of acute streptococcal infections.

Streptococcus pneumoniae is the most frequent cause of bacterial **lobar pneumonia** in humans. It is also a frequent cause of **otitis media** (infection of the middle ear) and **meningitis**. The bacterium colonizes the nasopharynx and from there gains access to the lung or to the eustachian tube. If the bacteria descend into the lung they can impede engulfment by alveolar macrophages if they possess a capsule which somehow prevents the engulfment process. Thus, encapsulated strains are able to invade the lung and are virulent (cause disease) and noncapsulated strains, which are readily removed by phagocytes, are nonvirulent.

The *Neisseriaceae* comprise a family of Gram-negative BetaProteobacteria with metabolic characteristics similar to pseudomonads. The neisseriae are small, Gram-negative cocci usually seen in pairs with flattened adjacent sides. Most neisseriae are normal flora or harmless commensals of mammals living on mucous membranes. In humans they are common residents of the throat and upper respiratory tract. Two species are primary pathogens of humans, *Neisseria gonorrhoeae* and *Neisseria meningitidis,* the bacterial causes of gonorrhea and meningicoccal meningitis.

Neisseria gonorrhoeae is the second leading cause of sexually-transmitted disease in the U.S., causing over three million cases of **gonorrhea** annually. Sometimes, in

females, the disease may be unrecognized or asymptomatic such that an infected mother can give birth and unknowingly transmit the bacterium to the infant during its passage through the birth canal. The bacterium is able to colonize and infect the newborn eye resulting **neonatal ophthalmia**, which may produce blindness. For this reason (as well as to control Chlamydia which may also be present), an antimicrobial agent is usually added to the neonate eye at the time of birth.

Neisseria meningitidis is one bacterial cause of meningitis, an inflammation of the meninges of the brain and spinal cord. Other bacteria that cause meningitis include *Haemophilus influenzae*, *Staphylococcus aureus* and *Escherichia coli*. **Meningococcal meningitis** differs from other causes in that it is often responsible for epidemics of meningitis. It occurs most often in children aged 6 to 11 months, but it also occurs in older children and in adults. Meningococcal meningitis can be a rapidly fatal disease, and untreated meningitis has a mortality rate near 50 percent. However, early intervention with antibiotics is highly effective, and with treatment most individuals recover without permanent damage to the nervous system.

Lactic acid bacteria are Gram-positive, nonsporeforming rods and cocci which produce lactic acid as a sole or major end product of fermentation. They are important in the food industry as fermentation organisms in the production of cheese, yogurt, buttermilk, sour cream, pickles, sauerkraut, sausage and other foods. Important genera are *Streptococcus* and *Lactobacillus*. Some species are normal flora of the human body (found in the oral cavity, GI tract and vagina); some streptococci are pathogens of humans (see pyogenic cocci above). Certain oral lactic acid bacteria are responsible for the formation of dental plaque and the initiation of dental caries (cavities).

Endospore-forming bacteria produce a unique resting cell called an **endospore**. They are Gram-positive and usually rod-shaped, but there are exceptions. The two important genera are *Bacillus*, the members of which are aerobic sporeformers in the soils, and *Clostridium,* whose species are anaerobic sporeformers of soils, sediments and the intestinal tracts of animals.

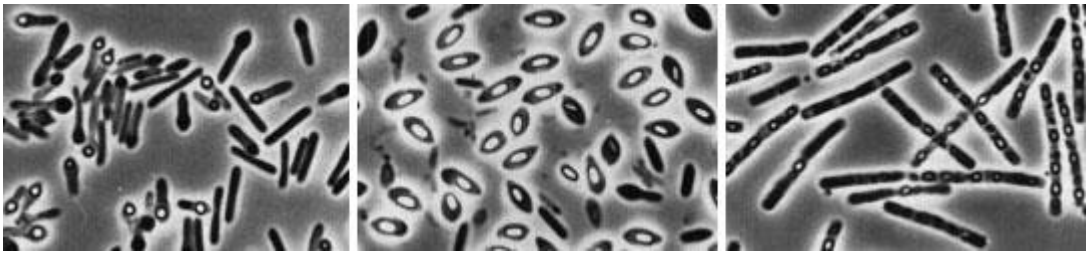


Figure 11. Endospore-forming bacilli (phase contrast illumination). Endospores are dehydrated, refractile cells appearing as points of bright light under phase microcsopy. Endospore-forming bacteria are characterized by the location (position) of the endospore in the mother cell (sporangium) before its release. The spore may be central, terminal or subterminal, and the sporangium may or may not be swollen to accomodate the spore.

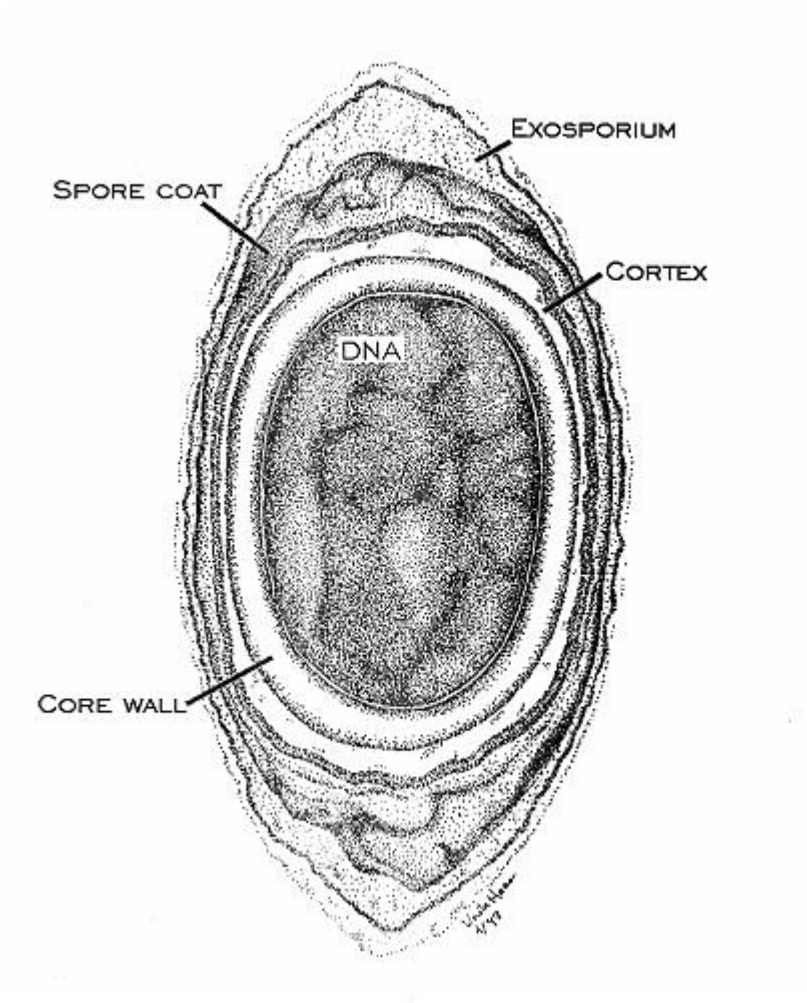


Figure 12. Anatomy of an endospore, cross section drawing by Viake Haas. Endospores differ from the vegetative cells that form them in a variety of ways. Several new surface layers develop outside the core (cell) wall, including the cortex and spore coat. The cytoplasm is dehydrated and contains only the cell genome and a few ribosomes and

enzymes. The endospore is cryptobiotic (exhibits no signs of life) and is remarkably resistant to environmental stress such as heat (boiling), acid, irradiation, chemicals and disinfectants. Some endospores have remained dormant for 25 million years preserved in amber, only to be shaken back into life when extricated and introduced into a favorable environment.

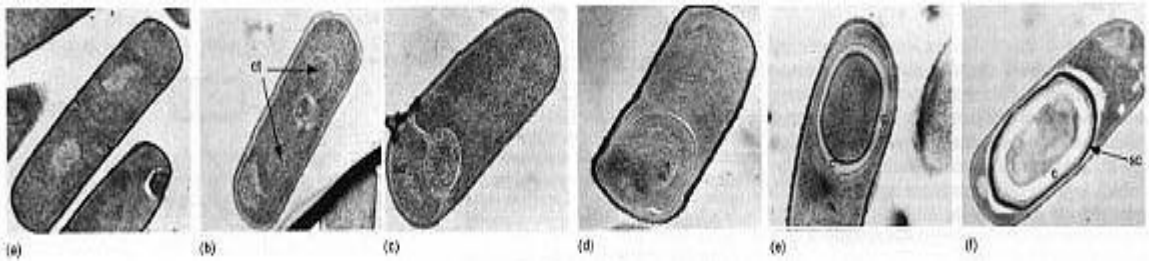


Figure 13. The sequential steps in the process of endospore formation in *Bacillus subtilis.*

Some sporeformers are pathogens of animals, usually due to the production of powerful toxins. *Bacillus anthracis* causes **anthrax**, a disease of domestic animals (cattle, sheep, etc.) which may be transmitted to humans. *Bacillus cereus* is becoming inceasingly recognized as an agent of food poisoning. *Clostridium botulinum* causes **botulism** a form of food-poisoning, and *Clostridium tetani* causes **tetanus**.

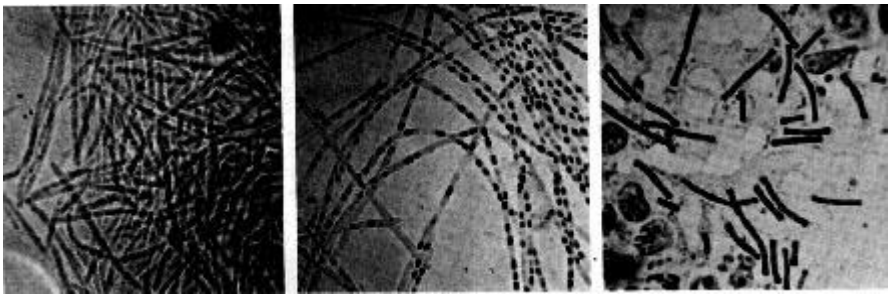


Figure 14. Robert Koch's original photomicrographs of *Bacillus anthracis.* In 1876, Koch established by careful microscopy that the bacterium was always present in the blood of animals that died of anthrax. He took a small amount of blood from such an animal and injected it into a healthy mouse, which subsequently became diseased and died. He took blood from that mouse and injected it into another healthy mouse. After repeating this several times he was able to recover the original anthrax organism from the dead mouse, demonstrating for the first time that a specific bacterium is the cause of a specific disease. In so doing, he established Koch's Postulates, which still today supply the microbiological standard to demonstrate that a specific microbe is the cause of a specific disease.

In association with the process of sporulation, some *Bacillus* species form a crystalline protein inclusion called **parasporal crystals**. The protein crystal and the

spore (actually the spore coat) are toxic to lepidopteran insects (certain moths and caterpillars) if ingested. The crystals and spores of *Bacillus thuringiensis* are marketed as "Bt" a natural insecticide for use on garden or crop plants. Another species of *Bacillus*, *B. cereus*, produces an antibiotic that inhibits growth of *Phytophthera,*, a fungus that attacks alfalfa seedling roots causing a "damping off" disease. The bacteria, growing in association with the roots of the seedlings, can protect the plant from disease.

Also, apparently in association with the sporulation process, some *Bacillus* species produce clinically-useful antibiotics. *Bacillus* antibiotics such as polymyxin and bacitracin are usually polypeptide molecules that contain unusual amino acids.

جامعة جنوب الوادى

كلية العلوم بقنا

الرؤيـــة

كلية العلوم بقنا تقدم خدمات تعليمية وبحثية ومجتمعية متميزة

الرســـالة

تلتزم كلية العلوم بقنا باعداد خريجين متميزين طبقا للمعايير الأكاديمية القومية وتقديم بحوث علمية متميزة وتطوير مهارات وقدرات الكوادر البشرية بها وتوفير خدمات مجتمعبة وبيئية تلبى طموحات كجتمع جنوب الوادى وذلك من خلال مشاركة مجتمعية فاعلة

ALL MY BEST WISHES

PROF. DR. WESAM M. A. SALEM