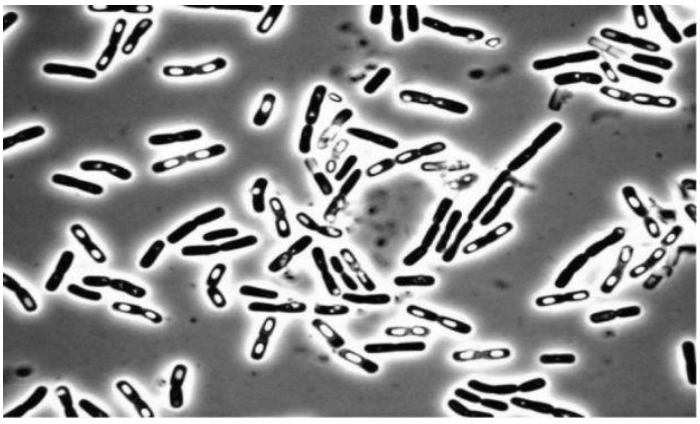
جامعة جنوب الوادى
كلية العلـــوم
قسم النبات والميكروبيولوجى

BACTERIOLOGY

for

Applied Microbiology Diploma



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Chapter (1)

OVERVIEW AND HISTORY OF BACTERIOLOGY

Bacteria are the earliest "residents" on Earth. Traces of them date back 3.5 billion years, whereas human beings have a history of only several millions of years. The tiny bacteria possess five characteristics that are impossible among higher organisms: (1) they have small volume but large surface area; (2) they absorb considerable nutrition and transform it quickly; (3) they are full of vitality, reproducing at high speed; (4) they constantly vary and are good at adapting; and (5) they have a great variety distributed in many places. They are everywhere. With proper methods, people can find these tiny fairies in almost all corners of the world. Bacteria are found in animals, plants, soil, water, and air, which can only be observed through microscopes. These bacteria include rumen bacteria in cows, *Bifidobacterium* in children's stomachs, cyanobacteria in oceans, enterobacter in birds' stomachs, sheath blight bacteria in ailed rice, and the oil-degraded microorganisms that consume petroleum.

Although not perceivable with the naked eye, bacteria leave their traces everywhere on Earth, from the flamboyant city to tranquil forest, from snow-covered mountains to vast seas, from the warm and rainy tropics to uninhabited deserts, from freezing polar zones to boiling volcanoes, from the surface of animals and plants to their insides, from dust floating in the sky to fossils lying

thousands of years underground. Bacteria have made their home everywhere.

Humans seem to live in a sea of bacteria. When you are playing or studying, there are numerous bacteria at your company; even in your tidy dorms, there are countless bacteria sharing the space with you. The environment we live in and the things we touch are often contaminated by bacteria, such as cleaning cloth, garbage bags, curtains, door handles, cutting boards, coins, public telephones, books and newspapers, tables and chairs, switches of electronic devices, bedroom furniture, and even some food. Research shows that a dirty hand carries 400,000 different bacteria with *Escherichia coli*, indicator of intestinal bacterial infection as the most abundant species.

BACTERIA IN HUMAN BODIES

Do not assume that bacteria will not like you, if you are a clean person; and do not assume that the less bacteria on your body the better. Scientific research shows that once you leave your mother's uterus, all types of bacteria attempt to "invade" each part of your body by various means, and they grow and regenerate, feeding on the nutrition in your body. Scientists speculate that on an adult body reside up to thousands of billions of bacteria, of more than 400 different types. The number of bacteria surpasses 90% of the total living cells of the human body (this includes all the living cells that make up the human body, as well as the microorganisms living inside the human body).

Bacteria's favorite camping places include mouth, nostrils, intestines, and the skin.

Bacteria are everywhere. The human body inevitably catches various bacteria, many of which are highly virulent, such as *Streptococcus*, *Staphylococcus aureus*, *Salmonella*, and *Mycobacterium tuberculosis*. Normally, harmful bacteria attach to the surface of human skin or hide inside the human body. When the bacteria multiply and exceed the defense capability of the human immune system, body resistance is low because of illness or weather changes, or a skin wound is infected, these bacteria will trigger disease. Furthermore, the normal flora we talk about is only nonpathogenic for individuals with corresponding immunity. Normal flora for one individual may be abnormal for another. For a given individual, immunity against normal flora is limited. An infection is triggered when the species' proportion or amount of normal flora change drastically, or when the flora invade normally bacteria-free places such as blood.

BACTERIA IN WATER

In the vast rivers, tranquil lakes, rough seas, gurgling brooks, and all other worlds of water lurk huge bacteria armies. As different water environments can offer different types and amount of supplies, the scale and forces of the bacteria "army" also varies. In lakes and ponds away from human activities, there are fewer bacteria because of a limited supply of nutrition (organic

substances) for the “army”; normally, each liter of water contains only a few dozen to hundreds of bacteria. Bacteria living in such environments are mainly *Pseudomonas fluorescens*, *Chromobacterium*, *Achromobacter*, sulfur bacteria, bacterial clothing, and iron bacteria. In lakes and ponds near cities and factories, there are more bacteria because of the presence of a large number of organic substances from domestic sewage and industrial wastewater polluting the water. Each milliliter of water is able to contain up to tens of millions or even hundreds of millions of bacteria. The main types of bacteria include *Bacillus*, *Proteus*, *Escherichia coli*, and *Streptococcus faecalis*, and sometimes even *Salmonella typhi*, *Shigella*, *Vibrio cholerae*, and other pathogenic bacteria. Pathogens in water primarily come from a variety of ill human and animal’s excrement. Normally, because water environment is not suitable for the growth and reproduction of pathogens, these bacteria can live only for several days to several weeks in water, and they cannot survive for a long time. However, because of the flow of water, these pathogens can still travel and cause epidemics.

In the vast expanse of the ocean, there stations a bacteria army with special skills. First of all, they are not afraid of salt. Every liter of seawater contains about 30 g of salt. Their second skill is resistance against enormous pressure. On plains, there is normally 1 atm of atmospheric pressure; but in oceans, thousands or even tens of thousands meters deep, the atmospheric pressure can

shoot up to thousands or tens of thousands of atm. Bacteria in the ocean can grow and reproduce under such extreme pressure. Their third skill is that they can withstand cold conditions. About 90% of seawater is below 5 °C. The fourth skill is living on little food. There is little nutrition in deep seas. Some types of planktonic marine bacteria have adapted to low nutrient seawater; once given rich nutrition in laboratory environments, they stop growing and reproducing instead. The ocean is always undergoing dramatic changes yet constantly remaining in equilibrium, always full of vitality. Marine bacteria play an important role here. When the dynamic balance of marine ecosystem is damaged, marine bacteria, with their sensitivity and adaptability, can multiply with great speed and form abnormal microbial flora in short time, actively participating in various activities to promote the formation of a new dynamic equilibrium. However, there are also many bacteria in the sea that are harmful to the human body. For example, there is a bacterium called the sulfate- reducing bacteria (SRB). It particularly likes to live on sailing boats. As many bacteria attach to the boat, they not only destroy the boat but also cost more power for navigation. As the saying goes, diseases enter by the mouth. As there are so many bacteria in water, we must ensure the quality of drinking water.

Quality standards of drinking water include three parts. First, to prevent epidemics, drinking water should not contain pathogenic microorganisms; second, to prevent acute and chronic

intoxication and potential long-term risks (carcinogenic, teratogenic, and mutagenic effects), chemical substances and radioactive materials in water should not threaten human health; and third, drinking water must ensure a good taste. As there are not many pathogens in water, and *Escherichia coli* and pathogens both come from fecal contamination of animals, one can check the number of *Escherichia coli* in water to determine the degree of water pollution by human and animal excrement, thus indirectly deduce the probability of the existence of other pathogens. According to China's drinking water health standards, the number of bacteria in each milliliter of water should not be more than 100; the number of *Escherichia coli* in each liter of water should not exceed 3.

BACTERIA IN SOIL

We often say soil is the "home base" of bacteria. Why? This is because it is very convenient for bacteria to eat and live there. One can say that a handful of soil is a colorful world of bacteria. Soil is rich in flora and fauna remains and in a variety of inorganic residues. It provides ample food for bacteria. Water and air in the soil can satisfy the needs of bacterial growth. Moreover, the pH of soil is close to neutral, and the temperature is generally constant all year round. It also has a suitable osmotic pressure for bacteria to grow. Therefore, in fertile soil, every gram contains billions of bacteria. Even in barren soil, each gram contains hundreds of millions of bacteria. There are hundreds of types of

bacteria in soil. Bacilli are the largest in number, followed by cocci. There are fewer vibrio and spirochetes. Spores of some pathogens such as *Bacillus anthracis*, *Clostridium tetani*, and *Botulinum* toxin can survive for a long time in soil. Therefore, soil can easily cause wound infections. Stationed in different soils, bacteria also develop different skills. Scientists can identify "special forces" of bacteria to serve human beings with their different skills. For example, they can find bacteria that live on oil in oil-rich areas, bacteria that decompose pollutants in discarded plastic bottles or plastic films in fields, and metal-eating bacteria in mining areas that can help people find gold.

BACTERIA IN THE AIR

Nutrition and water that is required for bacteria to live is lacking in the air. Meanwhile, ultraviolet light in the sun is the "mortal enemy" of bacteria. So why are there still bacteria in the air? In fact, there are numerous tiny dusts and water drops in the invisible and intangible air, which are the "hiding place" of bacteria. Meanwhile, some bacteria or bacterial spores with high resistance to ultraviolet and dryness can survive for a long time in the air. The number of bacteria varies according to the cleanliness of the air. Generally speaking, the more the dust is, the more the bacteria. There are more bacteria in the air over land than over an ocean; more in the air over cities than in rural areas; more in the air of dirty districts than in clean places; there are most bacteria in the air of places where the concentration of humans and animals

is high. Indoor air has more bacteria than outdoor air, especially in densely populated public places such as hospital wards and clinics. As dust falls naturally, the closer the air is to the ground, the more the bacteria.

Droplets, dander, sputum, pus, and feces carry numerous bacteria and can seriously pollute the air. Some medical procedures such as high-speed dental drill or ultrasonic cleaning can also cause air pollution; they produce aerosols for bacteria. Bacteria on fabric surfaces fly into the air when we dress or make the bed. The cleaning and the moving of people make dust float and are the main source of bacteria in hospitals. Therefore, people living in downtown areas need to visit the suburbs and parks more often to breathe fresh air. This will be of great benefit to their health. Especially in forest parks, the air is very clean and contains fewer bacteria, an ideal place for tourism, vacations, and convalescence.

There are no fixed types of bacteria in the air. Most of the time, bacteria in soil, humans, and animals float into the air with particles and dusts. Common bacteria in outdoor air include spore-producing bacilli and pigment-producing bacteria. The most common pathogens in indoor air include *Neisseria meningitidis*, *Mycobacterium tuberculosis*, hemolytic bacteria, diphtheria bacillus, pertussis, and others. Bacteria float in the air and enter human body with respiration. Therefore, modern public health standards emphasize a safe bacteria level in the air in public places, taking it as an important indicator of air

cleanliness. Scientists use PM2.5 to indicate air quality. PM2.5 refers to particles in the atmosphere with a diameter less than or equal to 2.5 μm (thinner than 1/20 of human hair). These particles are also known as inhalable particles. A higher PM2.5 measurement indicates more polluted air. Although PM2.5 constitutes only a small portion of the atmosphere, it bears great influence to air quality and visibility. In comparison to bigger particles, PM2.5 is small in size and rich in toxic and harmful material. They remain in the air for a long time and can be transported to distant places, so they have a greater impact on human health and atmospheric quality.

The main sources of PM2.5 are residues of emission from daily power generation, industrial production, and automobile exhaust; most of them contain heavy metal and other toxic chemicals. Generally speaking, coarse particles of 2.5–10 μm come primarily from road dust; particles below 2.5 μm (PM2.5) come primarily from the combustion of fuels (e.g., motor vehicle exhaust and coal burning). Nowadays, we often encounter grayish surroundings. We used to think it was just cloudy, but actually it is fog caused by high levels of PM2.5 and PM10.

THE GREAT DISCOVERERS

Originator: Leeuwenhoek

In 1632, Antonie van Leeuwenhoek was born in a small city in the east of the Netherlands called Delft. He did not receive any higher education, and he started apprenticeship in an Amsterdam

cloth shop at the age of 16. Later, he opened his own small cloth shop in Delft. Back then, people examine the quality of cloth with magnifying glasses. But Leeuwenhoek was not satisfied with examining his cloth with existing magnifying glasses. So he began to learn to make his own magnifying glasses.

Antonie van Leeuwenhoek (1632–1723).

In 1660, Leeuwenhoek got a new position as the administrator in Delft city hall. This was a leisurely job, so he had much more time to make magnifying glasses. Eventually, his glasses were achieving higher and higher magnification. Because the greater the magnification, the smaller the lens, Leeuwenhoek used two metal plates to sandwich the lens for convenience, and he attached a pointed metal stick to the front of the lens, so that things could be observed at the point. There was a screw knob to adjust the focus. He made up to 500 microscopes in his lifetime. Although they are more magnifying glasses with high powers rather than microscopes in its strict modern sense, but Leeuwenhoek observed raindrops, sewage, blood, pepper water, corrupt substances, alcohol, butter, hair, semen, muscle, tartar,

and many other substances. He also became the first person to have observed the world of microorganisms, which he called “tiny creatures”. He wrote more than 300 letters to scientists in the Netherlands and other countries. Some letters were translated by his friend Regnier de Graaf. de Graaf believed that more people should know about Leeuwenhoek’s work, so he urged Leeuwenhoek to contact the Royal Society of London for Improving Natural Knowledge. In 1676, Leeuwenhoek wrote a lengthy letter to the secretary of the Royal Society, reporting his observations of the past 20 years. He also included a picture of tiny creatures that people had never seen before — spherical, rod-shaped, and spiral-shaped bacteria and protozoa. Leeuwenhoek’s discovery was a sensation, and his letters in Dutch were translated into English or Latin and published in the journal of the Royal Society of London. Leeuwenhoek wrote 190 letters in total to the Royal Society and donated 26 microscopes. In 1680, he was elected a member of the Royal Society of London; in 1699, he was appointed the correspondent of the Academy of the Sciences in Paris; in 1716, he received a silver medal from the University of Leuven. Apart from observations through his microscopes and reporting his observations, Leeuwenhoek had no other hobbies. At the age of 90, 36 h before the end of his life, he was still writing to the Royal Society of London.

Leeuwenhoek is the founding father of bacteriology. However, in the next 200 years after Leeuwenhoek’s discovery, human

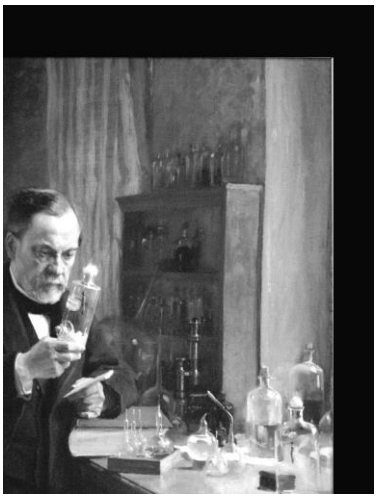
knowledge of bacteria was still limited to describing their forms and shapes. It was not until people used microscopes with higher magnification to reobserve Leeuwenhoek's "tiny creatures" and realized that bacteria can both cause disease and be beneficial did the greatness of Leeuwenhoek's contribution be fully appreciated.

Father of microbiology: Pasteur

Louis Pasteur was born in Dours, France, in 1822. His father was a cavalry officer under Napoleon. Since childhood, Pasteur had been determined to be a knowledgeable person. He set strict requirements for himself during school, trying to achieve perfection in every subject. In the summer of 1843, Pasteur entered Ecole Normale de Paris. Under the influence of then chemistry master J.B. Dumas, Pasteur devoted himself to the world of chemistry. Pasteur was conscientious, devoted, and did not work for fame or money. His professor believed such attitude to be the best reward for a teacher. At 25, Pasteur earned his PhD and remained a teaching assistant at his school. At 26, he discovered the principle of rotation, which at that time remained an unsolved question for many scientists. This principle opened new possibilities to research in stereochemistry, to which he was the founder.

Pasteur was one of the most accomplished scientists of the 19th century. He was acknowledged as the "father of microbiology" and the "most flawless man entering the kingdom of science". "Will, work, and success are the three elements of life. Will

opens the door to your career; work is the entering path; at the end of the path stands success to celebrate your hard work — so long as there is a strong will and hard work, there must be a day of success", this was Pasteur's famous slogan for success.



Louis Pasteur (1822–1895) in his lab.

Pasteur successfully researched and discovered vaccines for cholera, anthrax, and rabies, opening a new era of humans conquering infectious diseases. He laid the foundation of immunology, an important branch of science today.

Pioneer of bacteriology: Koch

Robert Koch was born in December of 1843, in Claudia Stelle near Hartz, Germany. In 1866, he graduated from the Medical Department of University of Gottingen. He then went to Berlin for 6 months of chemical research. In 1867, he became a resident doctor in Hamburg. During the Franco-Prussian War, he was the army surgeon. After the war, he opened a clinic in a town in eastern Prussia.

Robert Koch (1843–1910).

During his practice, he built a simple laboratory at home. Without equipment, access to libraries, or contact with other researchers, Koch started his research and accomplished many “firsts” in microbiology.

In 1876, he proved in a public presentation that anthrax bacillus was the pathogen of anthracis, which proved that certain bacteria caused certain diseases. Around 1881, he created microscopic photography technology and staining techniques to observe bacteria under microscopes.

In 1882, he invented the technique of separating and purifying microorganisms with solid agar medium.

In 1882, he isolated *Mycobacterium tuberculosis*.

In 1883, he isolated *Vibrio cholerae*.

In 1884, he established guidelines for identifying pathogenic microorganisms.

During 1891–1899, he discovered that rat flea is the media of plague, and trypanosomiasis is spread by tsetse fly.

In 1905, because of his contribution to tuberculosis research, Koch was awarded the Nobel Prize in Physiology or Medicine. All these accomplishments demonstrated the breakthroughs Koch had introduced to science and made him the tycoon in bacteriology.

On May 27, 1910 at the age of 67, Koch died of heart attackin Baden, Germany.In 1882, Koch discovered the pathogen that causes tuberculosis.He successfully separated *Mycobacterium tuberculosis* in solid serum medium. He replanted the bacteria in guinea pigs; they then developed tuberculosis. In 1883, Koch isolated and cultured *Vibrio cholerae* in India.

"Koch's postulates"

On the basis of his experience of isolating pathogens, Koch came up with the famous "Koch postulates": a pathogenic microorganism must be found in abundance in all organisms with the disease, but it should not be found in healthy organisms; the pathogenic microorganism must be isolated from a diseased organism and grown in pure culture; the cultured microorganism should cause disease when introduced into a healthy organism; the microorganism must be re-isolated from the inoculated and diseased experimental host and identified as being identical to the original specific causative agent.

These postulates are applied to identify whether a certain bacterium is a pathogen. The method is still used today and has made great contributions to human kind. With the guidance of

these postulates, the period between 1870s and 1920s was called the golden age of pathogenic discoveries. In 1883, diphtheria was found; in 1884, typhoid bacillus was found; in 1894, the plague bacillus was found; and in 1897, dysentery was found. Until 1990, 21 pathogens were discovered in the short 21 years. “With the right methods, discoveries will come like ripe apples falling from the tree”. And it was Koch who discovered this right method.

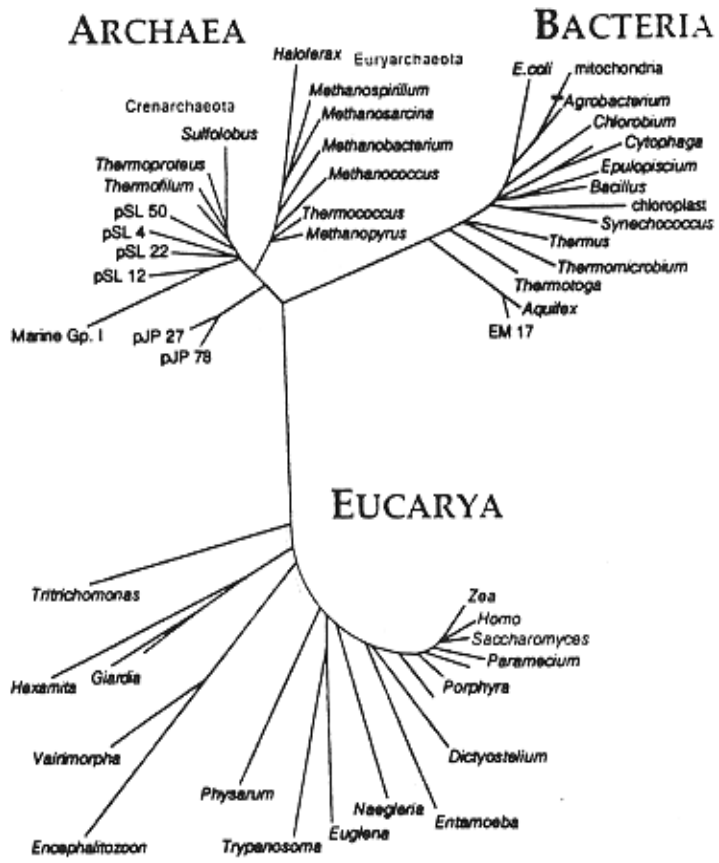
THE SCOPE OF BACTERIOLOGY

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 been renamed Archaea and has been awarded biological Domain status on the level with Bacteria and Eucarya.

THE UNIVERSAL TREE OF LIFE

On the basis of small subunit ribosomal RNA (ssrRNA) analysis the Woesean Tree of Life gives rise to three cellular "Domains":

Archaea, Bacteria, and Eucarya. Bacteria (formerly known as eubacteria) and Archaea (formerly called archaebacteria) share the procaryotic type of cellular configuration, but otherwise are not related to one another any more closely than they are to the eucaryotic domain, Eucarya. Between the two procaryotes, Archaea are apparently more closely related to Eucarya than are the Bacteria. Eucarya consists of all eucaryotic cell-types, including protista, fungi, plants and animals.



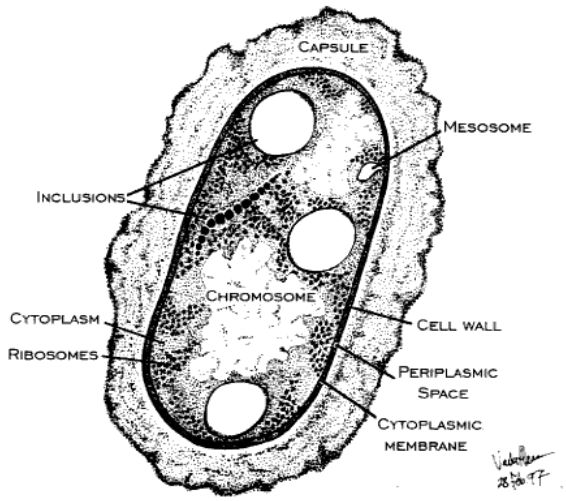
The Universal Tree of Life as derived from sequencing of ssrRNA. Note the three major domains of living organisms: Archaea, Bacteria and Eucarya. The "evolutionary distance" between two organisms is proportional to the measurable distance between the end of a branch to a node to the end of a comparative branch. For example, in Bacteria, *E. coli* is more closely related to *Agrobacterium* than to *Thermus.*

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 and archaea enter into various types of symbiotic relationships with plants and animals that usually benefit both organisms, although a few bacteria are agents of disease.

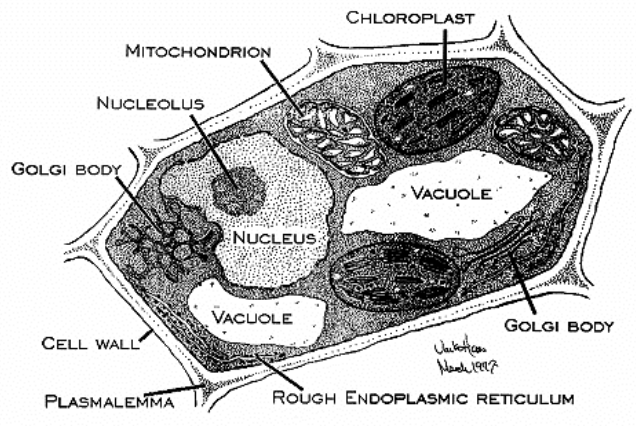
The metabolic activities of procaryotes in soil habitats have an enormous impact on soil fertility that can affect agricultural practices and crop yields. In the global environment, procaryotes are absolutely essential to drive the cycles of elements that make up living systems, i.e., the carbon, oxygen,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



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



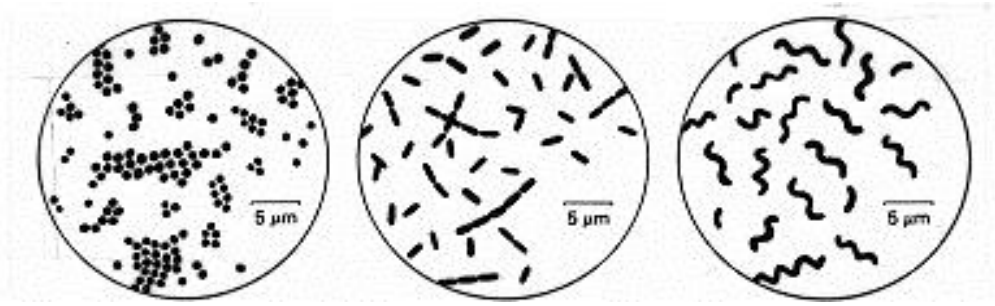
IDENTIFICATION OF BACTERIA

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.

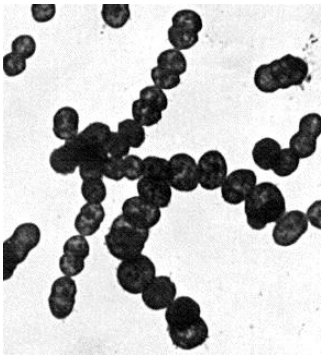
***Bacillus subtilis*, an abundant soil bacteria.**

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, followed by Bergey's Manual of Systematic Bacteriology, the "field guides" 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.



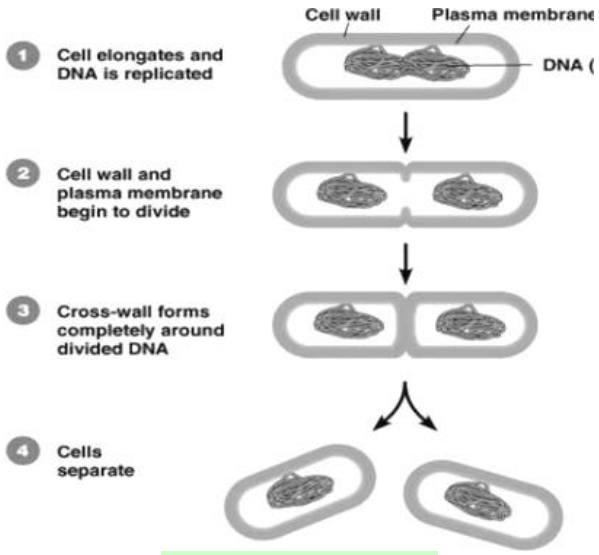
Size and fundamental shapes of procaryotes revealed by three genera of Bacteria (left to right): *Staphylococcus* (spheres), *Lactobacillus* (rods), and *Aquaspirillum* (spirals).



Chains of a dividing streptococci. Electron micrograph of *Streptococcus pyogenes*

BACTERIAL REPRODUCTION AND GENETICS

Most bacteria reproduce by a relatively simple asexual process called binary fission: each cell increases in size and divides into two cells.



Steps of binary fission

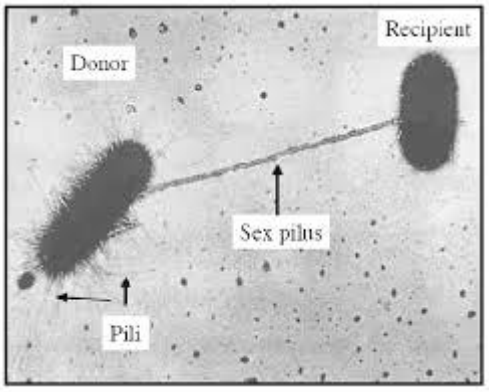
During this process there is an orderly increase in cellular structures and components, replication and segregation of the bacterial DNA, and formation of a septum or cross wall which divides the cell into two.

The time interval required for a bacterial cell to divide or for a population of 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.

Genetic exchange in Bacteria

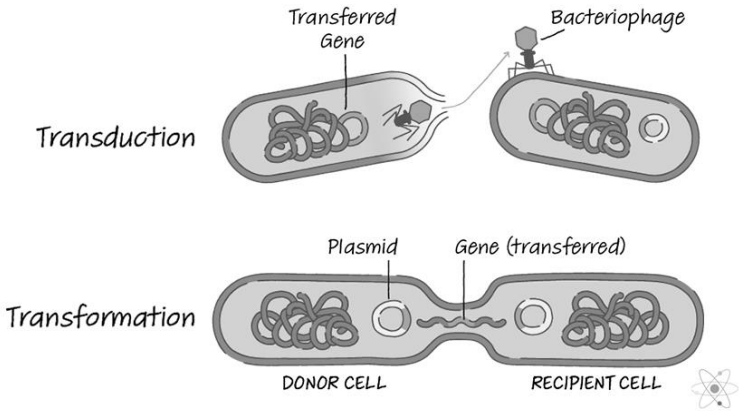
Although procaryotes do not undergo sexual reproduction, they are not without the ability to exchange genes and undergo genetic recombination. Bacteria are known to exchange genes in nature

by three fundamental 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.



Bacterial conjugation

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 genetic engineering of bacterial cells in the research or biotechnology laboratory is often based on the use of plasmids as vectors. The genetic systems of the Archaea are poorly characterized at this point.



Bacterial transduction and transformation

Evolution of Bacteria and Archaea

For most procaryotes, mutation is a major source of variability that allows the species to adapt to new conditions. The mutation rate for most procaryotic genes is in the neighborhood of 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 procaryotes 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. The evolution of procaryotes, driven by such Darwinian principles of evolution (mutation and selection) is called **vertical evolution**.

However, as a result of the processes of genetic exchange described above, the bacteria and archaea can also undergo a process of **horizontal evolution**. In this case, genes are transferred laterally from one organism to another, even between members of different Kingdoms, which allows the recipient to experiment with a new genetic trait. Horizontal evolution is

becoming realized to be a significant force in driving cellular evolution.

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 procaryotes.

ECOLOGY OF BACTERIA AND ARCHAEA

Bacteria and Archaea are present in all environments that support life. They may be free-living, or living in associations with "higher forms" of life (plants and animals), and they are found in environments that support no other form of life. Procaryotes have the usual nutritional requirements for growth of cells, but many of the ways that they utilize and transform their nutrients are unique. This bears directly on their habitat and their ecology.

Nutritional types of organisms

In terms of carbon utilization a cell may be heterotrophic or autotrophic. Heterotrophs obtain their carbon and energy for growth from organic compounds in nature. Autotrophs use $C0_2$ as a sole source of carbon for growth and obtain their energy from light (e.g. photoautotrophs) or from the oxidation of inorganic compounds (e.g. lithoautotrophs).

Most heterotrophic bacteria are saprophytes, meaning that they obtain their nourishment from dead organic matter. In the soil, saprophytic bacteria and fungi are responsible for biodegradation of organic material. Ultimately, organic molecules, no matter how

complex, can be degraded to CO_2 (plus H_2 and H_2O). Probably no naturally-occurring organic substance cannot be degraded by the combined activities of the bacteria and fungi. Hence, most organic matter in nature is converted by heterotrophs to CO_2, only to be converted back into organic material by autotrophs that die and nourish heterotrophs to complete the carbon cycle.

Lithotrophic procaryotes have a type of energy-producing metabolism which is unique. Lithotrophs (also called lithoautotrophs or chemoautotrophs) use inorganic compounds as sources of energy, i.e., they oxidize compounds such as H_2 or H_2S or NH_3 to obtain electrons to feed in to an electron transport system and to produce ATP. Lithotrophs are found in soil and aquatic environments wherever their energy source is present. Most lithotrophs are autotrophs so they can grow in the absence of any organic material. Lithotrophic species are found among the Bacteria and the Archaea. Sulfur-oxidizing lithotrophs convert H_2S to S and S to SO_4. Nitrifying bacteria convert NH_3 to NO_2 and NO_2 to NO_3; methanogenic archaea strip electrons off of H_2 as a source of energy and add them to CO_2 to form CH_4 (methane). Lithotrophs have an obvious impact on the sulfur, nitrogen and carbon cycles in the biosphere.

Photosynthetic bacteria convert light energy into chemical energy for growth. Most phototrophic bacteria are autotrophs so their role in the carbon cycle is analogous to that of plants. The planktonic cyanobacteria are the "grass of the sea" and their form

of oxygenic photosynthesis generates a substantial amount of O_2 in the biosphere. However, among the photosynthetic bacteria are types of photosynthetic metabolism not seen in eucaryotes, including photoheterotrophy (using light as an energy source while assimilating organic compounds as a source of carbon), anoxygenic photosynthesis, and unique mechanisms of CO_2 fixation (autotrophy).

Photosynthesis has not been found to occur among the Archaea, but one archaeal species employs a light-driven non photosynthetic means of energy generation based on the use of a chromophore called bacteriorhodopsin.

Adaptations to environmental conditions

Most procaryotes, whether they have been cultured and studied in the laboratory, or observed growing in their natural habitats, seem to be highly adapted to their specific environment by means of their macromolecular structure and/or their physiologic (metabolic) capabilities. The nutritional quality of the environment determines whether a particular organism will be present, but so do various physical parameters such as the availability of light and O_2, as well as the pH, temperature and salinity of the environment. As examples, the range of procaryotic responses to oxygen and temperature are discussed below.

Procaryotes vary widely in their response to O_2 (molecular oxygen). Organisms that require O_2 for growth are called

obligate aerobes; those which are inhibited or killed by O_2, and which grow only in its absence, are called **obligate anaerobes**; organisms which grow either in the presence or absence of O_2 are called **facultative anaerobes**. Whether or not a particular organism can exist in the presence of O_2 depends upon the distribution of certain enzymes such as superoxide dismutase and catalase that are required to detoxify lethal oxygen radicals that are always generated by living systems in the presence of O_2

Procaryotes also vary widely in their response to temperature. Those that live at very cold temperatures (0 degrees or lower) are called **psychrophiles**; those which flourish at room temperature (25 degrees) or at the temperature of warm-blooded animals (37 degrees) are called **mesophiles**; those that live at high temperatures (greater than 45 degrees) are **thermophiles**. The only limit that seems to be placed on growth of certain procaryotes in nature relative to temperature is whether liquid water exists. Hence, growing procaryotic cells can be found in supercooled environments (ice does not form) as low as -20 degrees and superheated environments (steam does not form) as high as 120 degrees. Archaea have been detected around thermal vents on the ocean floor where the temperature is as high as 320 degrees!

Symbiosis

The biomass of procaryotic cells in the biosphere, their metabolic diversity, and their persistence in all habitats that support life,

ensures that these microbe will play a crucial role in the cycles of elements and the functioning of the world ecosystem. However, the procaryotes affect the world ecology in another significant way through their inevitable interactions with insects, plants and animals. Some bacteria are required to associate with insects, animals or plants for the latter to survive. For example, the sex of offspring of certain insects is determined by endosymbiotic bacteria. Ruminant animals such as cows and sheep, whose diet is mainly cellulose (plant material), must have cellulose-digesting bacteria in their intestine to convert it to a form of carbon that the animal can assimilate. Leguminous plants grow poorly in nitrogen-deprived soils unless they are colonized by nitrogen-fixing bacteria which can supply them with a biologically-useful form of nitrogen.

Bacterial pathogenicity

Some bacteria are **parasites** of plants or animals, meaning that they grow at the expense of their eucaryotic host and may damage, harm, or even kill it in the process. Such bacteria that cause disease in plants or animals are **pathogens**. Human diseases caused by bacterial pathogens include tuberculosis, whooping cough, diphtheria, tetanus, gonorrhea, syphilis, pneumonia, cholera and typhoid fever, to name a few. The bacteria that cause these diseases have special structural or biochemical properties that determine their virulence or pathogenicity. These include: (1) ability to colonize and invade

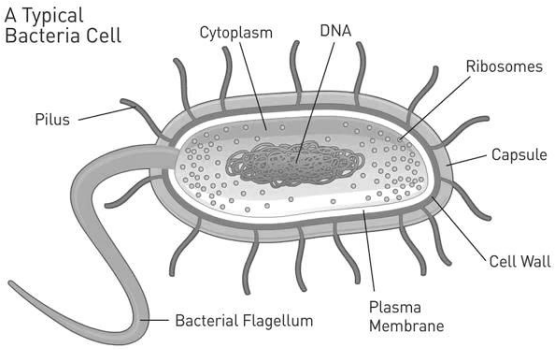
their host; (2) ability to resist or withstand the antibacterial defenses of the host; (3) ability to produce various toxic substances that damage the host. Plant diseases, likewise, may be caused by bacterial pathogens. More than 200 species of bacteria are associated with plant diseases, but a very small handful of genera are involved.

Chapter (2)

STRUCTURE AND FUNCTIONS 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.

Procaryotic cells have three architectural regions: appendages (proteins attached to the cell surface) in the form of flagella and pili; 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.



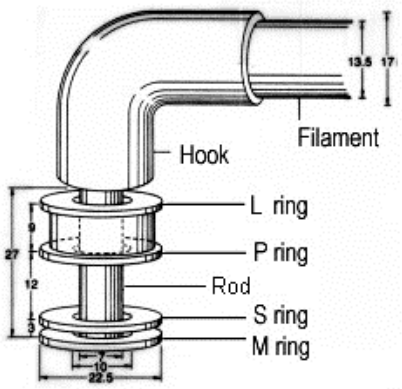
Characteristics of typical bacterial cell structures.

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

SURFACE STRUCTURES-APPENDAGES

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.



The ultrastructure of a bacterial flagellum. 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 filament rotates and contracts which propels and steers the cell during movement.

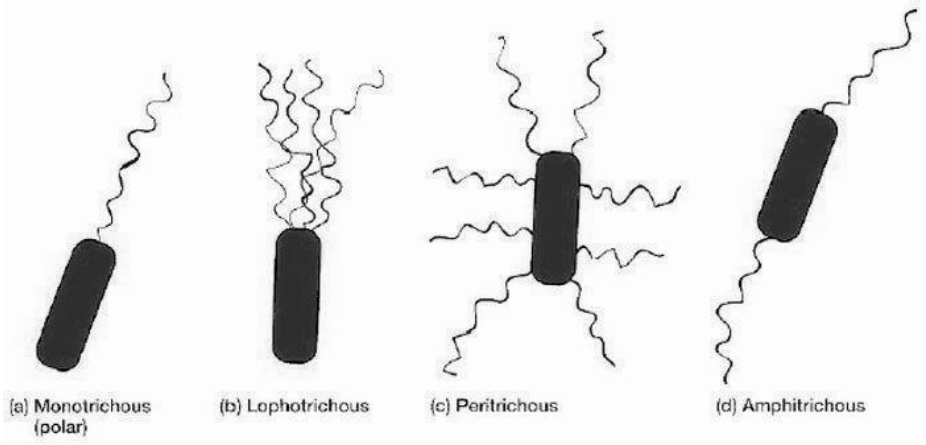
The ultrastructure of the flagellum of *E. coli* is illustrated in the Figure above. The flagellar apparatus consists of several distinct proteins: a system of rings embedded in the cell envelope (the basal body), a hook-like structure near the cell surface, and the flagellar filament.

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.

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.

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.

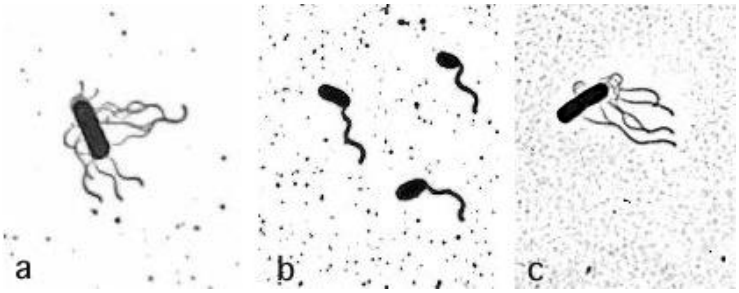


Types of bacterial flagellation

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.



Flagellar stains of three bacteria a. *Bacillus cereus* b. *Vibrio cholerae* c. *Bacillus brevis*. 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.

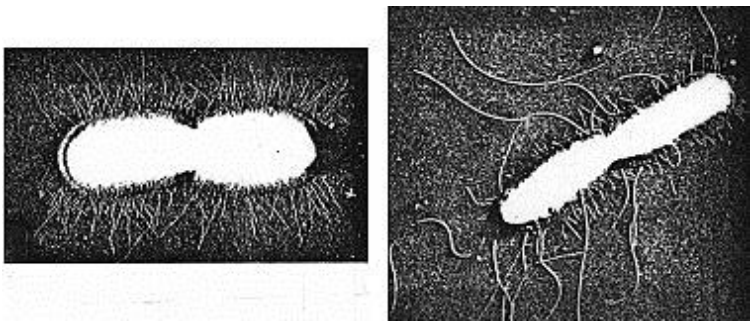
3. **direct microscopic observation** of living bacteria in a wet mount. One must look for transient movement of swimming bacteria. Most uincellular bacteria, because of their small size, will shake back and forth in a wet mount observed at 400X or 1000X. This is Brownian movement, due to random collisions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

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 such as the 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.



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. Both *Shigella* and *Salmonella* are enteric bacteria that cause different types of intestinal diarrheas. *Salmonella* is motile; *Shigella* is nonmotile.

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

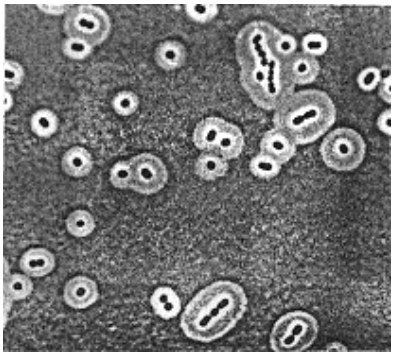
THE CELL ENVELOPE

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. 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.

Capsules

Most procaryotes contain some sort of a polysaccharide (or polpeptide) 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 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.



Bacterial capsules outlined by India ink viewed by light microscopy. This is a true capsule, a discrete layer of polysaccharide surrounding the cells.

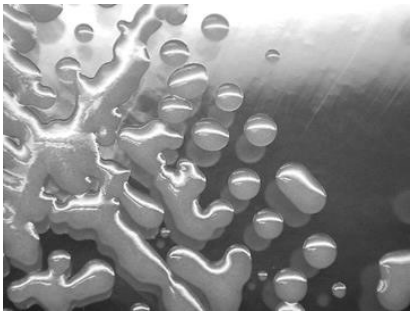
Functions of bacterial capsules

Capsules are generally composed of polysaccharide; rarely they contain amino sugars or peptides. 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.

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
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



Colonies of *Bacillus anthracis*. The slimy or mucoid appearance of a bacterial colony is usually evidence of capsule production. 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

.

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.

Biofilm

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*. 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.

THE 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. 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-

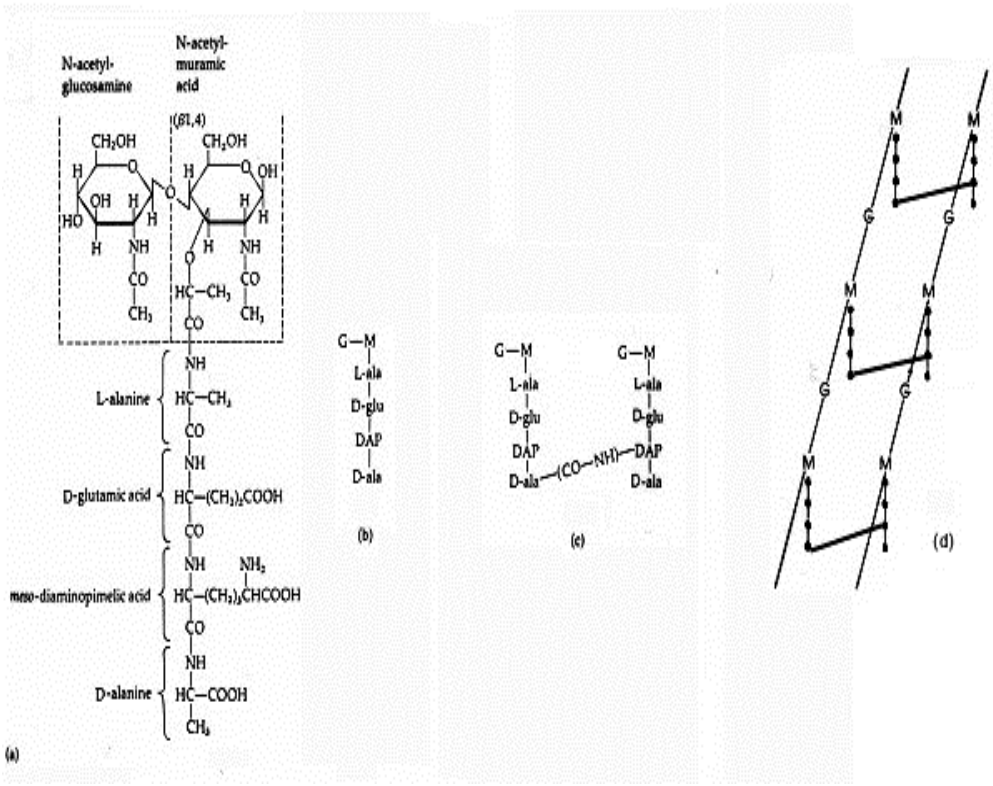
like molecules, but never do they contain murein. This feature distinguishes the Bacteria from the Archaea.

The profiles of the cell walls of Bacteria, as seen with the electron microscope, are redrawn in the following Figure. 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.

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 that connects the glycan backbone to a peptide side chain containing L-alanine, (L-ala), D-glutamate (D-glu), Diaminopimelic acid (DAP), and D-alanine (D-ala).

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.



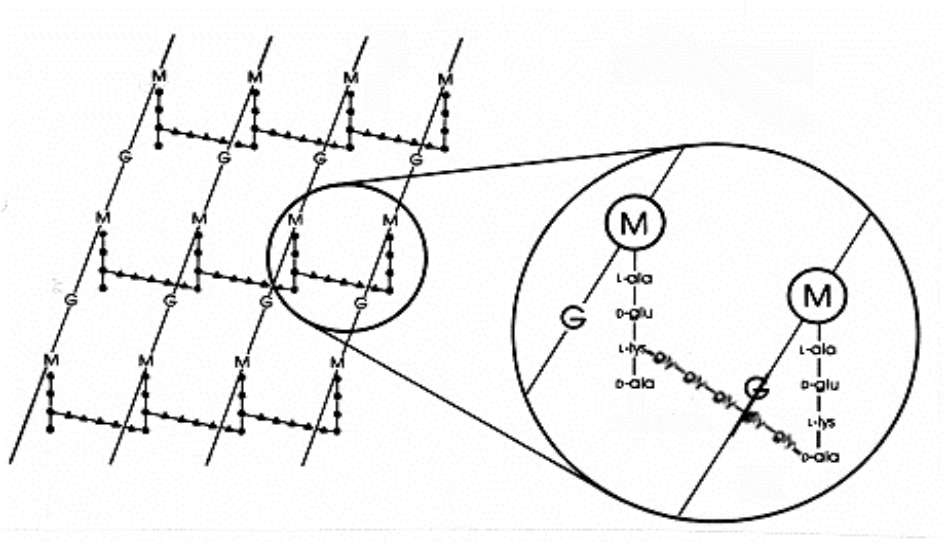
The structure of the muramic acid subunit of the peptidoglycan of *Escherichia coli*. This is the type of murein found in most Gram-negative bacteria: **a)** The glycan backbone is a repeat polymer of two amino sugars, N-acetylglucosamine (G) and N-acetylmuramic acid (M). Attached to the N-acetylmuramic 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.

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).

In Gram-positive bacteria there are numerous different peptide arrangements among peptidoglycans. The best studied is the murein of *Staphylococcus aureus*. 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. 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.



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.

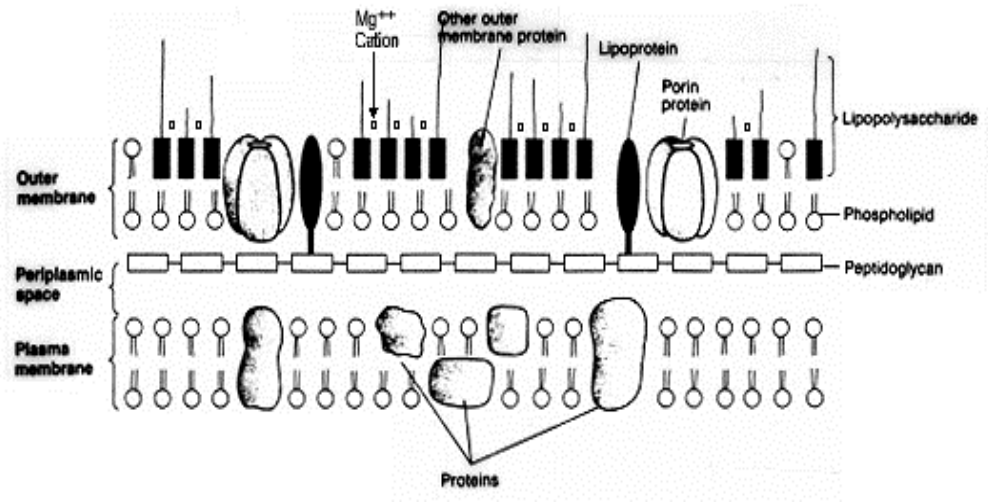
Gram-negative bacteria may contain a single monomolecular layer of murein in their cell walls while Gram-positive bacteria are thought to have several layers or "wraps" of peptidoglycan. Closely associated with the layers of peptidoglycan in Gram-positive bacteria are a group of molecules called teichoic acids. **Teichoic acids** are linear polymers of polyglycerol or polyribitol substituted with phosphates and a few amino acids and sugars. The functions of teichoic acid are not exactly known. There are

instances, particularly in the streptococci, wherein teichoic acids have been implicated in the adherence of the bacteria to tissue surfaces.

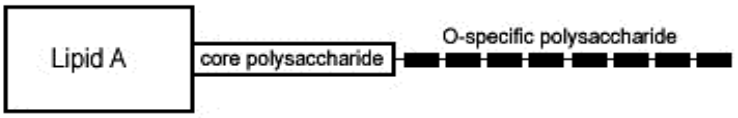
The outer membrane of Gm -ve Bacteria

It is a discrete bilayered structure on the outside of the peptidoglycan sheet (see the Figure below). The outer membrane is first 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.

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**.



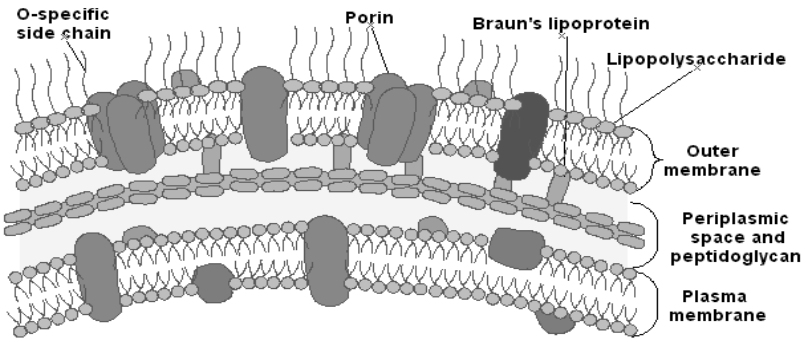
Schematic illustration of the outer membrane, cell wall and plasma membrane of a Gram-negative bacterium. Note the structure and arrangement of molecules that constitute the outer membrane.



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.

Gram-Negative Envelope



Bacterial lipopolysaccharides are toxic to animals. 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.

The proteins in the outer membrane of *Escherichia coli* are well characterized. 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. 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. Some proteins 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.

A correlation between Gram stain reaction and cell wall properties of bacteria is summarized in the following Table. The Gram stain procedure contains a "destaining" step wherein the cells are washed with solvent. 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.

Correlation of Gram 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 as 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.

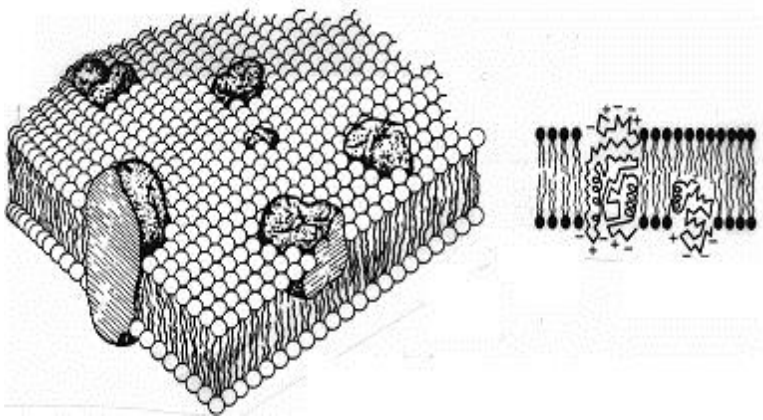
Functions of the procaryotic plasma membrane.

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

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.

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. The arrangement of proteins and lipids to form a membrane is called the **fluid mosaic model**, and is illustrated in the following Figure. The membranes of **Bacteria** are structurally similar to the cell membranes of eukaryotes, except that bacterial membranes consist of saturated or monounsaturated fatty acids (rarely, polyunsaturated fatty acids) and do not normally contain sterols.

The membranes of **Archaea** form bilayers functionally equivalent to bacterial membranes, but archaeal lipids are saturated, branched, repeating isoprenoid subunits that attach to glycerol via an ether linkage as opposed to the ester linkage found in glycerides of eukaryotic and bacterial membrane lipids. The structure of archaeal membranes is thought to be an adaptation to their existence and survival in extreme environments.



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 may form transport channels through the membrane.

CYTOPLASM AND ITS CONSTITUENTS

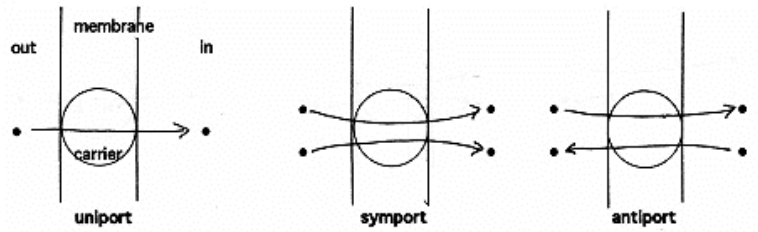
The cytoplasmic constituents of bacteria invariably include the procaryotic **chromosome** and **ribosomes**. The chromosome is typically one large circular molecule of **DNA**, more or less free in the cytoplasm, although intermittently associated with membranes. Procaryotes sometimes possess smaller extrachromosomal pieces of DNA called **plasmids**. The total DNA content of a cell is referred to as the cell **genome**. However, the eucaryotic processes of meiosis and mitosis are absent in procaryotes. Replication and segregation of procaryotic DNA is coordinated by the plasma membrane.

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 eucaryotes. Procaryotic ribosomes are 70S in size, being

composed of 30S and 50S subunits. The 80S ribosomes of eucaryotes 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 eucaryotes, Bacteria and Archaea.

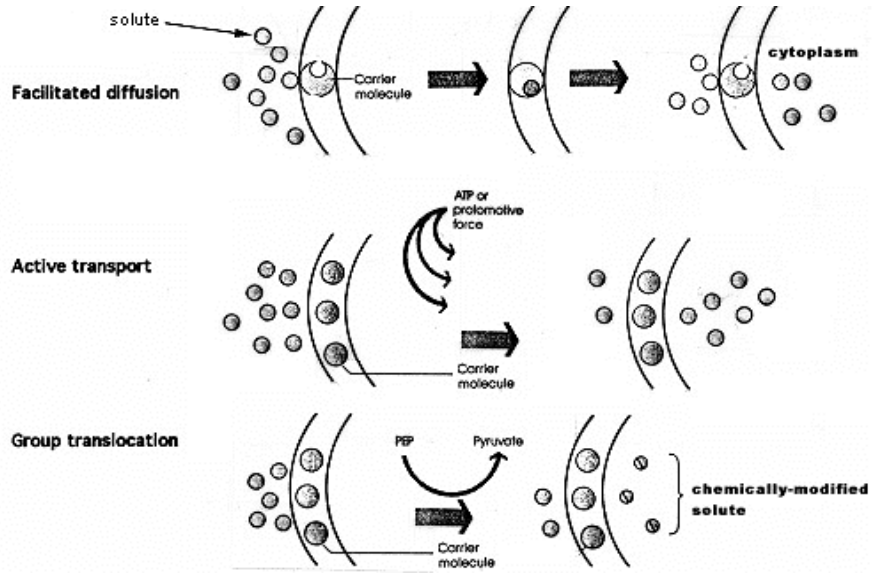
Often contained in the cytoplasm of procaryotic cells is one or another of some type of inclusion granules. **Inclusions** are distinct granules that may occupy a substantial part of the cytoplasm. Inclusion granules are usually reserve materials of some sort. For example, carbon and energy reserves 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 specialized enzyme complexes.

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 in the Figure below. In a **uniport** process, a solute passes through the membrane unidirectionally.



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 operate by one or another of these processes.

In **symport** processes 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. Bacteria have a variety of types of transport systems which can be used alternatively in various environmental situations as illustrated in the following Figure.



Operation of bacterial transport systems. **Facilitated diffusion** is a carrier-mediated system that does not require energy and does not concentrate solutes against a gradient. **Active transport** systems such as Ion-driven transport and Binding protein-dependent transport, use energy and concentrate molecules against a concentration gradient. **Group translocation** systems, such as the phosphotransferase (pts) system in *Escherichia coli*, use energy during transport and modify the solute during its passage across the membrane**.**

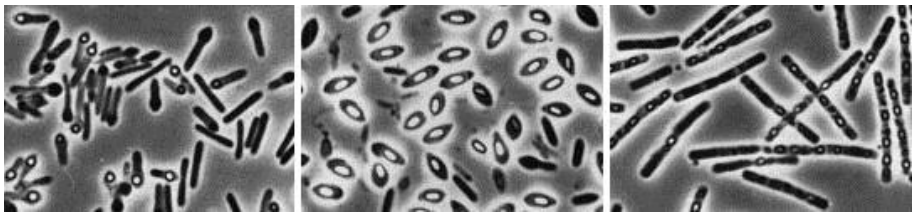
Most transport systems transport specific sugars, amino acids, anions or cations that are of nutritional value to the bacterium.

The plasma membrane of procaryotes may invaginate into the cytoplasm or form stacks or vesicles attached to the inner membrane surface. These structures are sometimes referred to as **mesosomes** Such internal membrane systems may be analogous to the cristae of mitochondria or the thylakoids of chloroplasts which increase the surface area of membranes to which enzymes are bound for specific enzymatic functions. The photosynthetic apparatus (light harvesting pigments and ATPase) of photosynthetic procaryotes is contained in these types of membranous structures. Mesosomes may also represent specialized membrane regions involved in DNA replication and segregation, cell wall synthesis, or increased enzymatic activity. There are a few antibiotics (e.g. polymyxin), hydrophobic agents (e.g. bile salts), and proteins (e.g. complement) that can damage bacterial membranes.

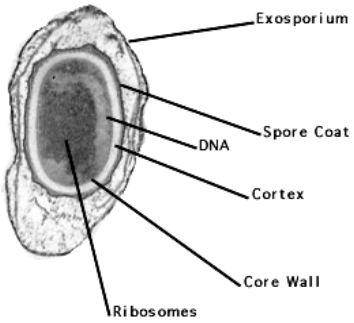
ENDOSPORES

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.



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



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..

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	Sensitive	Resistant

Chapter (3)

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 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. Major 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.

Trace Elements

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.

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 (PO_4)	Constituent of nucleic acids, nucleotides, 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

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 range of physical conditions such as O_2 concentration, temperature, and pH. Sometimes bacterial identification is based on the 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**.

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

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.

Some common vitamins required for 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
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

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.***

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 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 solid 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** is one in which the exact chemical composition is known. A **complex (undefined) medium** 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** 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.

Types of culture media:

1. **Synthetic of Defined Media**: Relatively simple media, all components are known

2. **Complex Media**: Composition of media not completely known. Often made from inexpensive organic materials such as slaughterhouse wastes (**tryptic** digests called tryptone, trypticase, etc.)

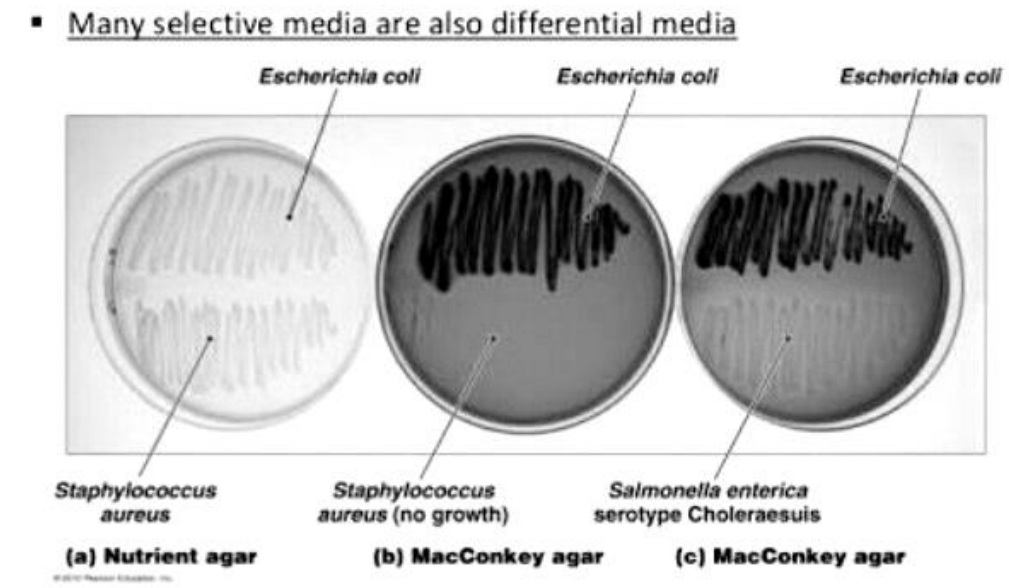
3. **Selective Media**: Media favors the growth of one or more microbes: Example: The use of bile salts to inhibit growth of most gram-positive bacteria and some gram-negative bacteria.

4. **Differential Media**: Media allows distinguishing between different bacteria that are growing. MacConkey agar has color indicator that distinguishes presence of acid which is a product of bacteria that ferment a particular sugar.

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.



PHYSICAL AND ENVIRONMENTAL REQUIREMENTS FOR BACTERIAL GROWTH

The procaryotes exist in nature under an enormous range of physical conditions such as O_2 concentration, Hydrogen ion concentration (pH) and temperature.

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.

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

Obligate anaerobes do not need or use O_2 as a nutrient. In fact, O_2 is a toxic substance that kills or inhibits their growth. Obligate anaerobic 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 they grow by fermentation or anaerobic respiration, but in the presence of O_2 they switch to aerobic respiration.

Terms used to describe O_2 relations of microorganisms.

Group	Environment		
	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

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.

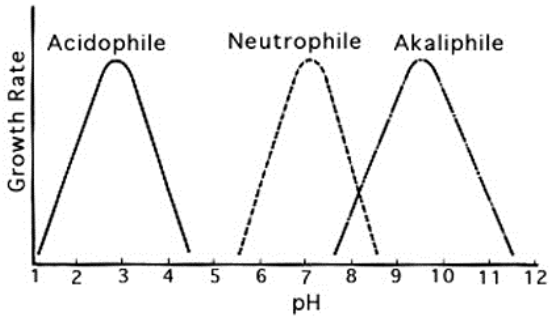
Effect of pH

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. 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.

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, require a low pH for growth since their membranes dissolve and the cells lyse at neutrality.

In the construction 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 and consider the 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.



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.

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

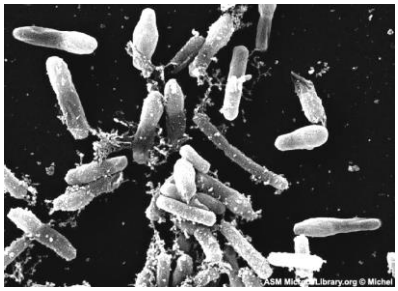
Organism	Minimum pH	Optimum pH	Maximum pH
Thiobacillus thiooxidans	0.5	2.0-2.8	4.0-6.0
Sulfolobus acidocaldarius	1.0	2.0-3.0	5.0
Bacillus acidocaldarius	2.0	4.0	6.0
Lactobacillus acidophilus	4.0-4.6	5.8-6.6	6.8
Staphylococcus aureus	4.2	7.0-7.5	9.3
Escherichia coli	4.4	6.0-7.0	9.0
Erwinia caratovora	5.6	7.1	9.3
Pseudomonas aeruginosa	5.6	6.6-7.0	8.0
Streptococcus pneumoniae	6.5	7.8	8.3

Effect of Temperature

Microorganisms have been found growing in virtually all environments where there is liquid water, regardless of its temperature.

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. 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 ºC (the body temperature of warm- blooded animals) are called **mesophiles**. Organisms with an optimum T between about 45 and 70 ºC are **thermophiles**. Some Archaea with an optimum T of 80 ºC or higher and a maximum T as high as 115 ºC, are referred to as **extreme thermophiles** or **hyperthermophiles**.

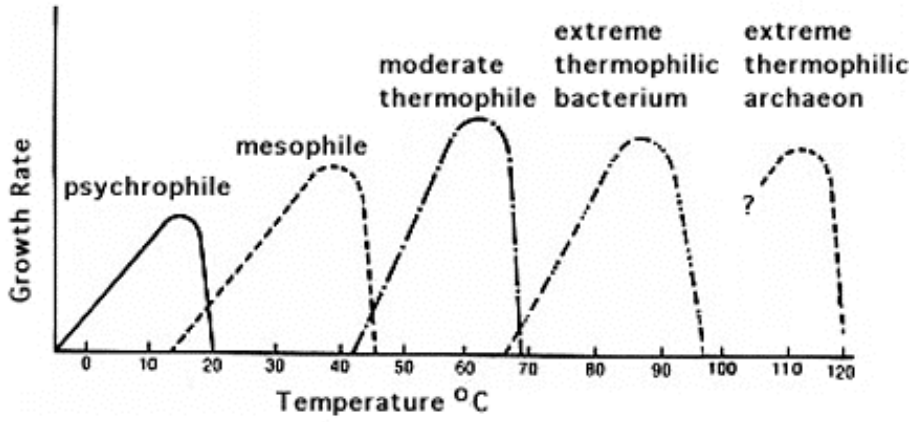


SEM (scanning electron microscopy) image of a thermophilic *Bacillus* species isolated from a compost pile at 55º C.

The cold- loving organisms are **psychrophiles** as they are able to grow at 0 ºC. A variant of a psychrophile (with optimum T of 10-15 ºC) is a **psychrotroph**, which grows at 0 ºC 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 ºC than at 25 ºC.

Think how fast milk spoils on the counter top versus in the refrigerator.



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.

Minimum, maximum and optimum temperature for growth of certain Bacteria and Archaea.

Bacterium	**Minimum**	**Optimum**	**Maximum**
Listeria monocytogenes	1	30-37	45
Pseudomonas maltophilia	4	35	41
Thiobacillus novellus	5	25-30	42
Staphylococcus aureus	10	30-37	45
Escherichia coli	10	37	45
Streptococcus pyogenes	20	37	40
Streptococcus pneumoniae	25	37	42
Bacillus flavothermus	30	60	72
Thermus aquaticus	40	70-72	79
Sulfolobus acidocaldarius	70	75-85	90

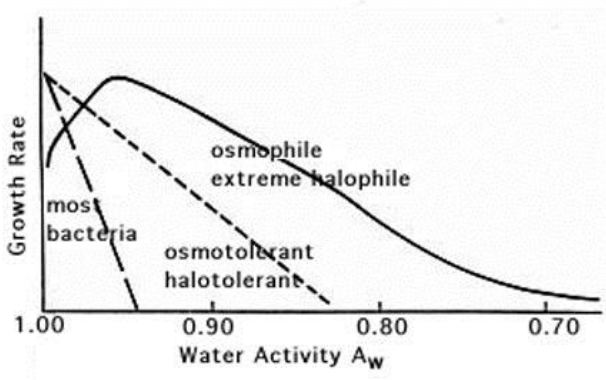
Water Availability

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 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.



Growth rate vs osmolarity for different classes of procaryotes. Osmolarity is inversely related to water activity (A_w). Increased solute concentration means increased osmolarity and decreased A_w. From 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 of an extreme halophile such as the archaean *Halococcus.* Note that a true halophile grows best at salt concentrations where most bacteria are inhibited.

Limiting water activities (A_w) for growth of certain procaryotes.

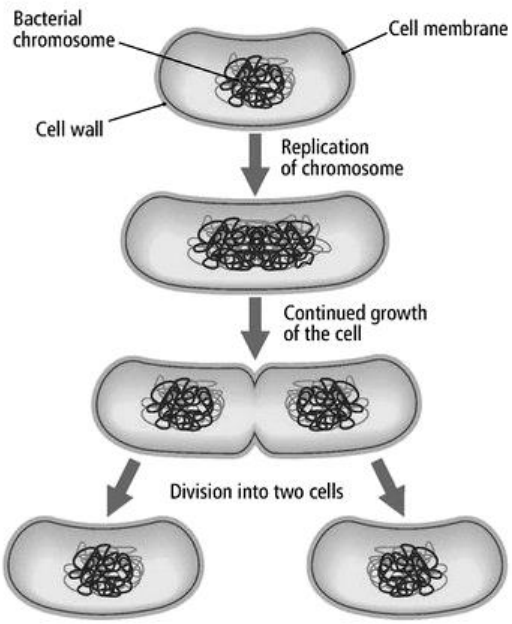
Organism	**Minimum A_w for growth**
Caulobacter	1.00
Spirillum	1.00
Pseudomonas	.91
Salmonella/E. coli	.91
Lactobacillus	.90
Bacillus	.90
Staphylococcus	.85
Halococcus	.75

Chapter (4)

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**.



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.

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**.

Measurements 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 (packed cell volume).

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.

Measurements 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.

2. **Electronic counting chambers** count numbers and measure size distribution of cells. 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 gastrointestinal tract.

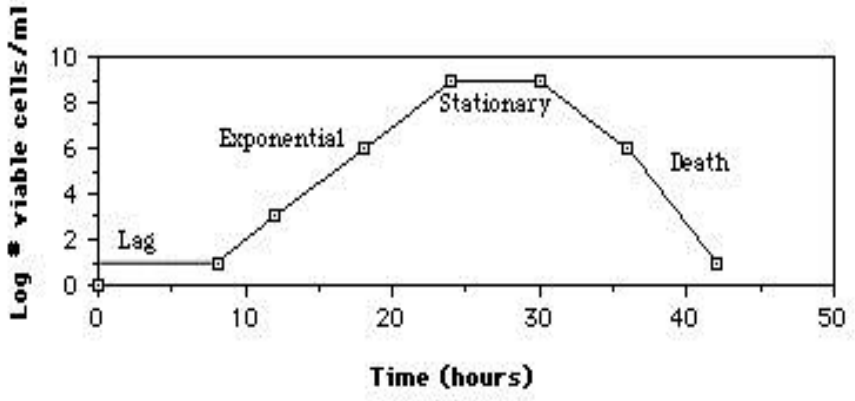
Some Methods used to measure bacterial growth

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 3 below).



The typical bacterial growth curve. 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 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 etc.

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. 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 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 is determined that some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing. 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 is during the stationary phase that spore- forming bacteria have to activate 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. Generation times for a few bacteria are are shown in the following Table.

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

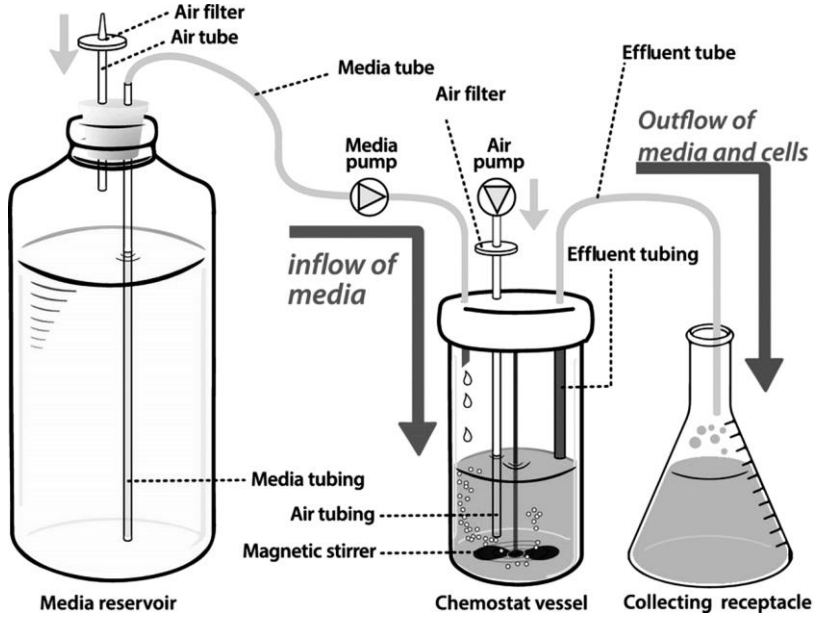
Bacterium	Medium	Generation Time (minutes)
Escherichia coli	Glucose-salts	17
Bacillus megaterium	Sucrose-salts	25
Streptococcus lactis	Milk	26
Streptococcus lactis	Lactose broth	48
Staphylococcus aureus	Heart infusion broth	27-30
Lactobacillus acidophilus	Milk	66-87
Rhizobium japonicum	Mannitol-salts-yeast 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** (see the following Figure), 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, 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 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.



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**.

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. Therefore, synchronous growth of a population of bacterial cells is obtained mechanically by filteration or chemically by adding/ or omitting nutrients or growth factors. Synchronous cultures rapidly lose synchrony because not all cells in the population divide at exactly the same size, age or time.

Chapter (5)

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 effected 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 then is one of these concepts to control microorganisms by different methods. Sterilizaton 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

(1) 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^{o}C for 30 minutes. Kills everything except some endospores (Actually, for the purposes of purifying drinking water 100^{o}C 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^{o}C 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^{o}C/ 2 hours or 170^{o}C/ 1 hour 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 the following Table.

(2) 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.

Recommended use of heat to control bacterial growth

Treatment	Temperature	Effectiveness
Incineration	>500^{o}	Vaporizes organic material on nonflammable surfaces but may destroy many substances in the process
Boiling	100^{o}	30 minutes of boiling kills microbial pathogens and vegetative forms of bacteria but may not kill bacterial endospores
Intermittent boiling	100^{o}	Three 30-minute intervals of boiling, followed by periods of cooling kills bacterial endospores
Autoclave and pressure cooker (steam under pressure)	121^{o}/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^{o}/2 hours	For materials that must remain dry and which are not destroyed at T between 121^{o} and 170^{o} Good for glassware, metal, not plastic or rubber items
Dry heat (hot air oven)	170^{o}/1 hour	Same as above. Note increasing T by 10 degrees shortens the sterilizing time by 50 percent
Pasteurization (batch method)	63^{o}/30 minutes	kills most vegetative bacterial cells including pathogens such as streptococci, staphylococci and Mycobacterium tuberculosis
Pasteurization (flash method)	72^{o}/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

(3) <u>Filtration</u>: 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.)

(4) 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. Methods include:

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^{o}C/ 30minutes; flash method: 71^{o}C/ 15 seconds.

Low temperature (refrigeration and freezing): Most organisms grow very little or not at all at 0° C. 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, evapouration, 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 and may have a static or cidal effect.

TYPES OF ANTIMICROBIAL AGENTS

Antiseptics: microbicidal agents harmless enough to be applied to the skin and mucous membrane; should not be taken internally. 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, copper sulfate, quaternary ammonium compounds.

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

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) is not.

Preservatives: static agents used to inhibit the growth of microorganisms, most often in foods. If eaten they should be nontoxic.

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 useful in the treatment of microbial or viral disease including **Antibiotics** that are 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 cell types. 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.

Chapter (6)

BENEFICIAL AND HARMFUL BACTERIA

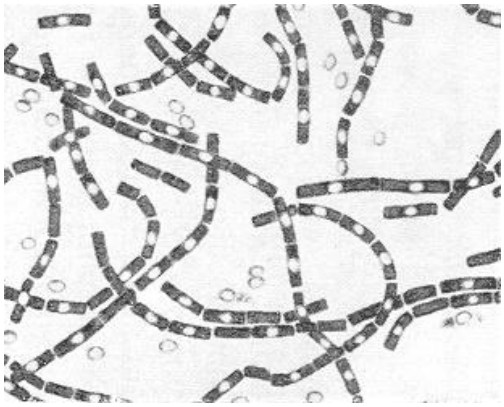
How can you find out if the microorganism is beneficial or not? One way to find out is to see the outcome of its action on organic matter. Beneficial microorganisms cause fermentation while harmful or pathogenic microorganisms cause putrefaction. Fermentation is a process by which useful substances such as alcohol, amino acids, organic acids and antioxidants are produced. These substances are useful to man, plants, and animals. Putrefaction, on the other hand, is a process by which harmful substances such as hydrogen sulfide, foul smell due to mercaptan, ammonia, and oxidants are produced. Food poisoning can result from ingestion of these products. On the other hand, the disease-causing microorganisms, especially bacteria, are almost well known. Frtunately, these are represented by a little number of bacteria compared to the huge numbers of beneficial ones.

EXAMPLES OF HARMFUL BACTERIA

Anthrax

Bacillus anthracis is the first pathogen discovered in human history. It is one of the biggest pathogenic bacilli with two flat ends. There are no flagella; however, but there are spores. The name of anthrax is derived from coal. It was named after the typical black skin of patients. It is an acute infectious disease

caused by *Bacillus anthracis*, which affects both humans and animals.



Bacillus anthracis containing spores

Early symptoms include spots on the face, neck, shoulders, hands, feet, and other exposed areas on the skin, which feel itchy. This is followed by blisters and hemorrhagic necrosis, which later develop into ulcers. Afterward, blood secretions form black dry scabs like charcoals. Under the scab, granulation tissues form anthrax carbuncles. The scab falls off and forms a scar. During the disease, patients experience symptoms such as fever, headache, swollen local lymph nodes, and splenomegaly. In some cases, the condition is serious enough to cause circulatory failure and, subsequently, death. If pathogens enter into the blood, they can cause sepsis as well as pneumonia and meningitis.

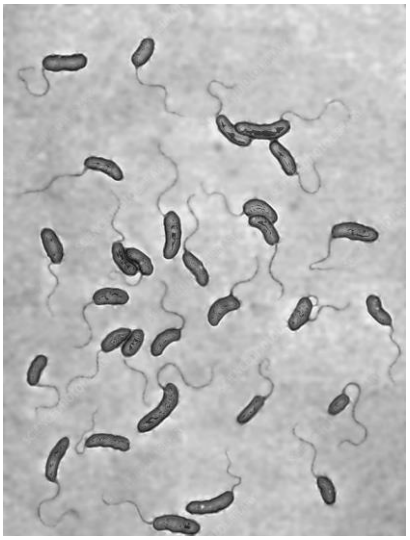
Pulmonary anthrax is caused by inhaling anthrax spores. Clinical manifestations include chills, fever, shortness of breath, difficulty in breathing, wheezing, cyanosis, blood sputum, and chest pain. Patients are often in serious conditions, accompanied by sepsis and septic shock and occasionally by meningitis.

Cholera

In less than 20 years, cholera has become the most horrifying and notable disease of the 19th century. In the 100 years until 1923, there had been six pandemics of cholera, the damage of which was innumerable. In India alone, more than 38 million people died. During the fifth pandemic in 1883, Koch discovered *Vibrio cholerae* from the feces of an Egyptian patient for the first time. After 1961, a seventh pandemic broke out.

Cholera is a highly infectious disease caused by *Vibrio cholerae*. *Vibrio cholerae* bacteria are curvy, like an arc or a comma. On one end of the bacteria is single flagellum and pili.

Patients show symptoms such as vomiting and diarrhea. The diarrhea effluent looks like water left behind after rinsing rice and contains considerable amount of *Vibrio cholerae*. Serious dehydration and salt loss may cause metabolic acidosis. Blood circulation may fail, and the patient can suffer shock or death.



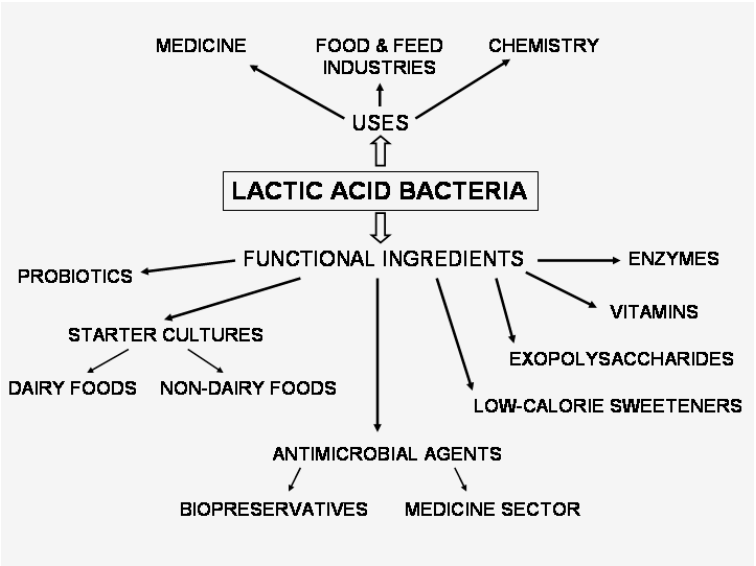
Vibrio cholera with flagella

EXAMPLES OF BENEFICIAL BACTERIA

Among those beneficial microorganisms are the following:

Lactic acid bacteria

As the name means, lactic acid bacteria (LAB) produce lactic acid, usually from sugars or other carbohydrates. Many lactic acid bacterial strains are used in dairy industry for the production of various kinds of cheeses, yogurt, butter and other food products.



Various uses and products of lactic acid bacteria

Recently, LAB have captured growing research attention because of their role in maintaining gut health. LAB have shown a positive effect in preventing cancer in the colon. The exact mechanism of control of colon cancer by LAB is currently unknown although there are several possible mechanisms including:

• modifications of the metabolic activities of the intestinal microflora and the physico-chemical conditions in the colon

• the ability to bind and degrade potential carcinogens;

• quantitative and/ or qualitative alterations in the intestinal microflora;

• production of antitumourigenic or antimutagenic compounds;

• enhancing the host's immune response; and/ or

• effects on the physiology of the host.

Bacteriocin production

Several microorganisms are capable of inhibiting or reducing contamination caused by spoilage and pathogenic microorganisms, through the production of various antimicrobial agents such as diacetyl, carbon dioxide, peroxide, bacteriocins and ethanol. Many studies have been conducted in the production of bacteriocins by Gram- positive bacteria, particularly lactic acid bacteria. Many of these bacteriocin- producing bacteria are already widely used in the food industry. An interesting point about bacteriocins is their potential application in the prevention of infectious diseases and to control biofilm formation.

Bacteriocins are ribosomal peptides that differ from other non-ribosomal peptides with antimicrobial activity in one critical feature that the former have a relatively narrow killing spectrum, as they are generally only toxic to bacteria closely related to the producing strain. Many bacteriocins are produced by food- grade lactic acid bacteria, a phenomenon which offers food scientists the possibility of directing or preventing the development of specific bacterial species in food. This can be particularly useful in preservation or food safety applications.

Lactic acid bacteria and their antimicrobial metabolites have potential as natural preservatives to control the growth of spoilage and pathogenic bacteria in foods. To date, nisin is the only bacteriocin that has found practical applications in some industrially processed foods. Its antibacterial activity and possible use as a biopreservative has been studied in a large number of food systems. Its application for the control of some pathogens and food spoilage organisms has been approved in a number of countries. Lactobacilli continue to remain the most commonly used probiotic microorganisms. Currently available probiotic preparations contain *Lactobacillus delbreuckii sp. bulgaricus, L. acidophilus, L. casei, L. fermentum, L.plantarum, L. brevis, L. lactis and L. Reuteri* [

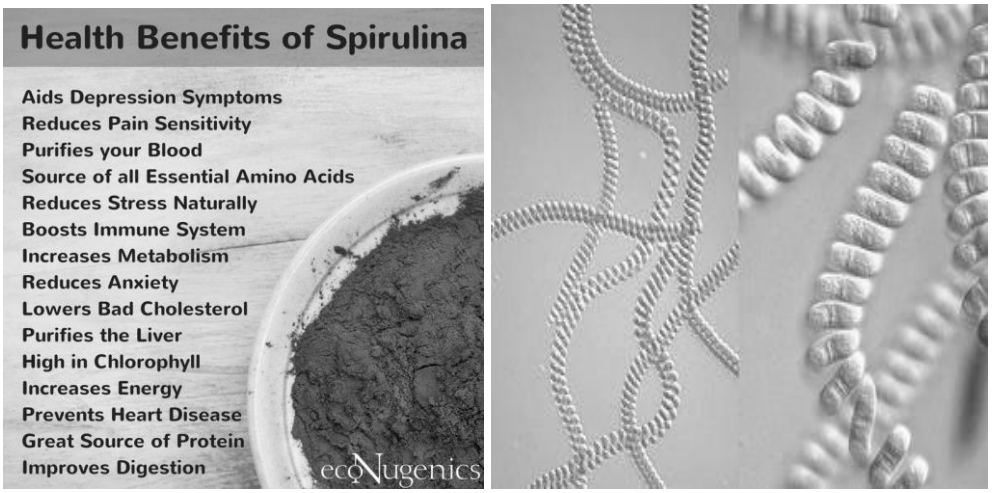
Bacteriocins are extremely effective candidates as therapeutic agents showing potential benefits in the treatment of cancer. Bacteriocins kill other bacteria present in the same environment that might compete for space and nutrients.

Medical benefits of Cyanobacteria

Cyanobacteria, a group of Gram- negative oxygenic autotrophs, are one of the oldest and most successful life forms present on the Earth comprising more than 150 genera and 2000 species. Cyanobacteria represent a great bioresource for a diverse range of secondary metabolites including biologically active compounds. These compounds show anti-algal, antifungal, antibacterial,

antiviral, antilarval, antiprotozoan, antihelmintic, antimitotic, anticancer, anticoagulation, and hemagglutinating properties.

For example, *Spirulina* is a good natural cancer-fighting agent. Moreover, supplementation of *Spirulina* spp. has helped in stabilizing blood sugar, reducing diabetes risk, reducing inflammation, and in preventing eye diseases.



Ten health benefits of Spirulina powder (right) and the organism under light microscope (left)

BACTERIAL PRODUCTS FOR HUMAN HEALTH

Hyaluronic acid

Hyaluronic acid (HA), a biological polymer, has long been known. HA is applied in ophthalmology, and new applications are found in surgery, drug delivery, orthopaedics and joint operations, cardiovascular medicine, and in cosmetics. HA is a highly viscoelastic glycosaminoglucan- polymer that forms part of the connective tissue in all vertebrates, where it lubricates the joints and acts as a "shock absorber". This substance is also responsible

for the maintenance of the semi- elliptical form of the human eye and the consistence of the aqueous humor.

HA is also a component of the capsule occurring in certain *Streptococcus* bacteria. This has led to HA production through fermentation. Shiseido Co. has been producing HA since 1985 through fermentation with the capsule- forming bacterium *Streptococcus zooepidemicus*. Today HA is marketed by Biomatrix (U.S.A.), Pharmacia (Sweden), and Shiseido Co. (Japan) and other companies. Novozymes has recently announced the low- cost production of very pure high molecular weight HA that makes use of a *Bacillus* organism, a significant improvement over the use of the (pathogenic) *Streptococcus* organisms. In the cosmetics sector, the sodium salt of HA with a molecular weight of 1.1 to 1.6 million daltons is used as an ingredient of ointments, lotions, and makeup for skin care: it protects and lubricates the cells, helps in the transport of certain molecules, and controls moisture retention.

Fucogel®

Recently, it has been demonstrated that the slimy capsular polysaccharide material, rich in the rare sugar L-fucose and produced by bacteria such as *Klebsiella* sp. and *Clavibacter* sp., can act as an efficient skin moisturizer. When applied as a film, it has a perfect psychosensorial touch and lowers the allergic response. Such a polysaccharide from *Klebsiella* sp. is marketed

under the name of Fucogel® and is also an excellent formulation agent.

Xanthan and other exopolysaccharides

Xanthan is industrially produced through fermentation with the bacterium *Xanthomonas campestris* (20,000 ton/ year). Xanthan is a biopolymer (which is formed extracellularly) with a b-1,4-glucan backbone. The combination of various unique physicochemical properties (pseudoplastic rheology, viscosity independent of pH, temperature, and salt concentration, etc.) renders this biopolymer extremely useful for numerous applications. By adding xanthan to paints, pesticides, detergents, fire- extinguishing agents, pharmaceutical preparations, explosives, printing inks for paper and fabrics, cosmetics, and so on, the viscosity, flocculation, jellifying, and rheological behavior can be controlled. Xanthan is marketed by Merck & SD- Kelco Inc., USA and others. In the cosmetics sector, it is applied in toothpaste and as an emulsion and suspension stabilizer and waterbinder in ointments, lotions and shampoos, and after- sun preparations. A mixture of xanthan gum and locust bean gum (St. John's bread) is also used in deodorant gels. Another exopolysaccharide (EPS) with molecular weight is produced by the bacterium *Rhizobium meliloti*. It forms a gel at low concentrations and does not precipitate in the presence of ethanol or polyethylene glycol and stabilizes emulsions that contain up to 50% oil. Its favorable moisturizing, jellifying, and thickening

properties allow many applications in medicine, pharmacy, and cosmetics.

Sunless tanning and sunlight protection agents

Dihydroxyacetone (DHA) is a type of sugar that has a strongly reactive keto group. It is a white, crystalline powder with a sweet, cool taste. It is highly soluble in water, alcohol, acetone, and ether. However, one of the most widely known commercial applications of DHA is in cosmetics, where it is used as an active component in self- tanning lotions.

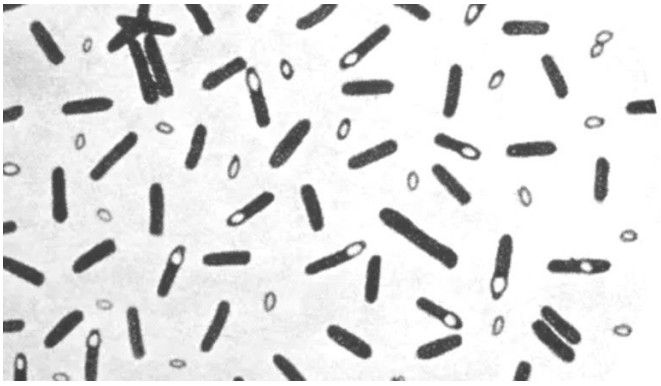
The formation of DHA through microorganisms from glycerol was already established in 1898. Since then, a great deal of research into this microbial fermentation process has been conducted, which is nowadays carried out on a commercial basis. The bacterium *Gluconobacter oxydans* is used industrially today for the production of DHA from glycerol. Chemical synthesis copes with difficulties and with a lower yield. Malyltyrosine is also a "fast-tanning agent." Its synthesis is carried out by a *Micrococcus caseolyticus* protease, which links malic acid and L-tyrosine (produced microbiologically) through a peptide bond.

Urocanic acid is a UV-B absorbing agent which is proper to the skin and is formed in the epidermal cells from L-histidine. Urocanic acid is now also produced microbiologically from the amino acid L-histidine, which is a fermentation product itself. This bioconversion is conducted commercially in Japan with the

Achromobacter liquidum ammonialyase. It is used in suntan products that offer protection against UV-B.

Botox for medical and cosmetic use

The protein toxin produced and excreted by the anaerobic, endospore- forming soil bacteria *Clostridium botulinum* is probably the most poisonous molecule known on earth. It is known under the name of botox. This bacterial species was discovered and studied in 1895 by Prof. E. Van Ermengem, microbiologist at Ghent University, Belgium. The toxin can cause food poisoning, named botulism, through the eating of food that is contaminated with the bacteria or the toxin. The toxin migrates to the muscles and nerves, where it results in the weakening of muscles, dizziness, difficulty in breathing, paralysis, and ultimately death.



Clostridium botulinum under light microscope with terminal endospores

This "botox" toxin can however be used medically, when applied in very low doses, as a "medicine". In 1989, National Institutes of Health (NIH) experts and the U.S. Food and Drug Administration (FDA) gave permission to its therapeutic use, in nanogram doses.

Nowadays it is used medically to cure focal dystonia around the neck, jawbone, vocal cords, tennis elbow or to stop migraine. It has made many operations and other medications unnecessary. However, repeated topical injections of the toxins remain necessary. Unfortunately, some patients develop antibodies to the toxin, which makes the desired effect disappear.

Other (potential) medical applications are the weakening of muscles that control the activities of the stomach and the intestines or to remedy the paralyses that are the consequence of a stroke, and so on. As there are different types of this neurotoxin, for a few years now, neurotoxin A is being used in the cosmetic sector to make wrinkles (crow's feet, etc.) disappear in the skin (by relaxing skin muscles) or to counter excessive sweating and hyperhydrosis, and so on. Also there are very recent medical indications that long- term use of botox can lead to headaches, nausea, and heart disorders, and that after a cosmetic facial treatment with botox injections, new wrinkles may appear. The botox toxin is produced through industrial fermentation processes with the appropriate *Clostridium botulinum* strains or this toxin is subsequently transformed into a vaccine.

Antibiotics

Some microorganisms fight each other, one producing certain substances to restrain or kill the other. This is called "antagonism", which is especially common among microorganisms. Microorganisms with antagonistic capabilities

are called antagonistic bacteria. Antibiotics are the weapons that antagonistic bacteria use to battle with other microorganisms. The first antibiotic used in clinical treatment of bacteria- infected disease was penicillin. Although it was produced from molds, the blooming scientific research based on its discovery reveals that some bacteria are also “masters” at producing antibiotics. Bacitracin produced by *Bacillus subtilis* and polymyxin produced by *Bacillus polymyxa*, for example, can interact with cell walls and led useful substances to flow out of the cell, thus killing the cell. Meanwhile, actinomycetes in microorganisms produce the most types of antibiotics. Most commonly in clinical use, streptomycin, gentamicin, and kanamycin are called aminoglycoside antibiotics; erythromycin, spiramycin, and midecamycin are called macrolide antibiotics; tetracycline, oxytetracycline, and chlortetracycline are called tetracycline antibiotics; and daunorubicin and doxorubicin are called anthracycline antitumor antibiotics. These are all weapons created by actinomycetes to defeat the enemies.

Steroid hormones

Various steroid hormones, such as cortisone, used to treat pain and inflammation, and progesterone, used to prevent miscarriages, can be synthesized commercially by microorganisms. The process involves providing an appropriate microorganism with a commonly available sterol compound that it can convert into the medically valuable hormone. Such minor chemical alterations by

bacteria and fungi, transforming a common substrate into a valuable product, are known as bioconversions.

BACTERIAL METABOLITES OF NONMEDICAL USES

Enzymes

Microorganisms produce a large variety of novel enzymes, a number of which have industrial applications. For example cardiologists use the bacterial enzyme streptokinase to dissolve clots in the arteries of heart attack patients. Also, stonewashed jeans have more to do with bacterial enzymes and less to do with "stones" than the name implies. Likewise, proteases in drain cleaners help to digest clogs caused by hair, which is largely protein. They also have a role in laundry detergents, where they digest protein- containing stains, such as blood stains. A grass stain, on the other hand, often is dissolved by bacterial cellulases in the detergent, which digest the cellulose in the plant material into simple sugars that are rinsed away.

A newer and potentially exciting group of enzymes is the **extremozymes**, produced by thermophilic or other microorganisms normally found in extreme environments. Thermophiles, for example, thrive in very hot water and their enzymes are capable of activity at temperatures that would normally inactivate typical enzymes. Polymerase chain reaction (PCR), so vital in modern genetic analysis and biotechnology, involves a series of high- temperature steps. Most DNA polymerase enzymes would quickly be denatured and would be of

no use at these temperatures. Accordingly, PCR relies on a type of DNA polymerase known as Taq polymerase. The enzyme is named after the thermophilic bacterium *Thermus aquaticus*, from which it is obtained. Many industrial processes that work best at high temperature might benefit from such heat- tolerant enzymes. Likewise, other procedures might require a very cold, acidic, or halophilic environment. Enzymes produced by psychrophilic (cold- loving), acidophilic (acid- loving), or halophilic (salt-loving) bacteria may then be most appropriate.

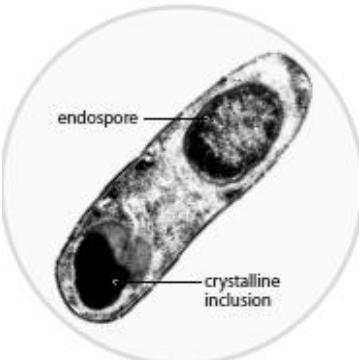
Organic acids and solvents

The sour taste of oranges, grapes, and lemons is the result of the various organic acid substances they contain. Some bacteria can produce organic acids. *Acetobacter* and *Gluconobacter*, for example, can produce vinegar; *Lactobacillus* and *Leuconostoc* can produce lactate; *Arthrobacter paraffineus* and *Corynebacterium* can produce citric acid; and Corynebacteriaceae and *Pseudomonas* can produce gluconate. Organic solvents such as ethanol, butanol, and glycerol are important raw materials for many industrial products. Some are also important fuels. *Clostridium* can produce butanol through fermentation. Other bacteria and yeasts are used to convert glucose in plants into ethanol (bioethanol) that will be discussed later.

Biopesticides

Bacillus thuringiensis is a spore- forming bacterium producing a protein that is highly toxic to many types of insects, particularly

the moth larvae that can cause harm to tomatoes, and other commonly cultivated plants. A subspecies, *Bacillus thuringiensis israelensis*, is especially effective against mosquito larvae and is often used in mosquito control programs. Spores containing the protein toxin are ingested by the insect, and after the cells lining the gut are destroyed, the toxin enters the insect's blood, causing paralysis and death. The protein is most lethal against insects that have a basic pH in their digestive system. Fortunately, dogs, cats, and other animals, as well as humans, have either a neutral or acidic pH in their digestive system, making the toxin safe to use.

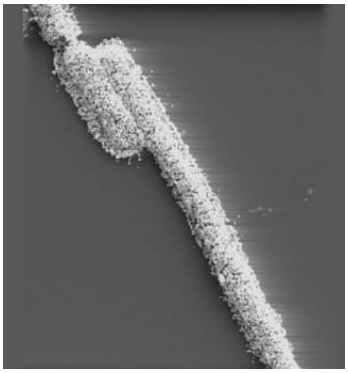


***Bacillus thuringiensis*: the microbial source of the biological pesticide Bt.** The bacillus has already formed its spore. Such spores contain a protein, which is toxic to many insect pests. *B. thuringiensis* is grown in fermentors, and when the bacteria have reached high numbers, conditions within the fermentor are altered to induce spore formation. The spores can then be sold as Bt, which can be used on plants to reduce insect damage.

After the bacteria are grown in large numbers in a fermentor, the conditions are altered to induce spore formation. The spores are then collected and mixed with inert compounds, for marketing as a pest control agent that can be dusted on plants. This "biopesticide" is often referred to simply as "Bt".

Bacteria in detectors and biosensors

Increasingly, bacteria are being considered not just for what they produce but as materials themselves. For example, researchers have converted bacteria into humidity detectors by coating the bacteria with tiny gold particles. As the environment dries out and humidity falls, the bacteria lose water, moving the gold particles on their surface closer together. Electrodes apply a voltage across the bacteria, and as the gold beads get closer together, an increased current is detected. The scientists found that if the humidity dropped from 20% to 0%, there was a high increase in current.



Microbial humidity sensors. The bacteria in the photograph, *Bacillus cereus*, have been coated with microscopic gold particles. Electrodes can then apply a current, transferred by the gold particles. As the humidity drops, the bacteria lose water, reducing their volume and bringing the gold particles closer together. This is detected as an increase in current.

These microscopic barometers, which function even if the bacteria die, work best in dry environments. The researchers have even suggested that they might be useful on future space missions to detect humidity on other planets such as Mars. Similar metal-plated microorganisms were used as semiconductors, magnets,

and optical devices, and for use in other nanotechnology applications.

APPLICATIONS FOR ENVIRONMENTAL POLLUTION

Sewage treatment, a bioremediation process

The rotten-egg smell in sewage treatment plants comes from hydrogen sulfide, released by bacteria as they digest organic material in the sewage. Sewage plants often combat this problem by using devices called chemical scrubbers that filter the hydrogen sulfide through lye and bleach. Although this technique works, it is at best a partial solution, because new environmental problems are created in the process. Researchers at the University of California, Riverside, however, may have come up with a cheaper, more environmentally sustainable strategy replacing the scrubbers with hydrogen sulfide- digesting bacteria. The sewage is trickled through biofilms of the bacteria, growing on polyurethane foam in a process known as **bioremediation**.

Microbial biofiltration. Foam blocks on which the bacteria grow as a biofilm. As sewage trickles through the foam, the bacteria convert the hydrogen sulfide into odorless hydrogen sulfate.

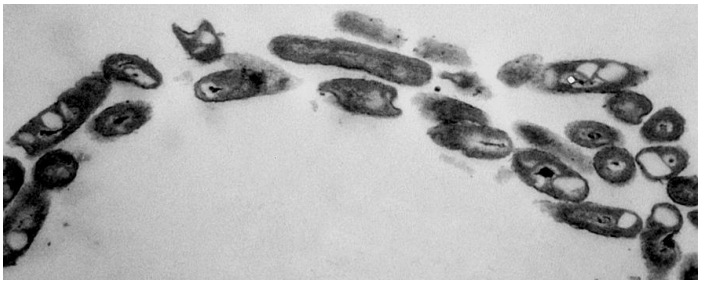
The microorganisms convert the offending hydrogen sulfide into odorless hydrogen sulfate, which is carried away by water seeping over the foam. The technique has already been instituted at several California treatment plants, where it costs about $50,000 to make the switch from scrubbers to bioremediation. Plants then save about $30,000 a year in operating costs.

Bacteria in soil remediation

Currently, bioremediation is the main technology of remediating polluted soil. Bioremediation technology is the process where microorganisms “eat” organic pollutants in the soil and convert them into carbon dioxide and water or other harmless substances. As natural bioremediation process is slow, industrialized bioremediation is introduced. It is an artificially promoted remediation. The degradation capacity of microorganisms can eliminate petroleum or other toxic and harmful pollutants from soil. Degradation can be realized through modification of physical and chemical conditions of soil (temperature, humidity, pH, aeration, and nutritional additives); it can also be achieved by inoculating specially engineered microorganisms that can increase the degradation rate. Remediation of organic- polluted soil with microorganisms is based on degradation and conversion of pollutants. It includes aerobic and anaerobic processes. In aerobic processes, microorganisms can degrade organic pollutants in soil and convert them into carbon dioxide and water; in anaerobic

processes, the main products are organic acids and others (methane and hydrogen, for example).

Scientists planted some special bacteria in the soil polluted by oil leaked from a nearby oil storage tank; they also put some nutrients in the soil at the same time. It turned out that in less than 60 days, these bacteria consumed 80% of the oil in the soil. Scientists emphasize that, compared to burning, cleaning, and other methods of freeing soil from oil pollution, the cost of biological methods is lower. Soil contamination by oil is regional; this makes it easier to apply biological method for oil removal.



Oil eating bacteria

BACTERIA AND THE RENEWABLE ENERGY

Bioethanol

Ethanol is a biological fuel (biofuel) already in widespread use. It is produced by fermenting agricultural products such as corn or sugarcane and then frequently blended with gasoline for use in motor vehicles. Ethanol, however, is not without problems. Ethanol, when made from corn, diverts an important food item from livestock feed or the dinner table to ethanol production plants. With so much corn being used for fuel, the price we pay for food has risen accordingly. Consequently, there has been

renewed interest in "cellulosic ethanol," for which plant wastes such as stalks and leaves are the source instead of edible plant parts such as corn kernels. Many microorganisms are able to digest cellulose, and if these microbes were used in ethanol fermentation, it might be possible to use plant wastes as a sugar source.

Biomethane

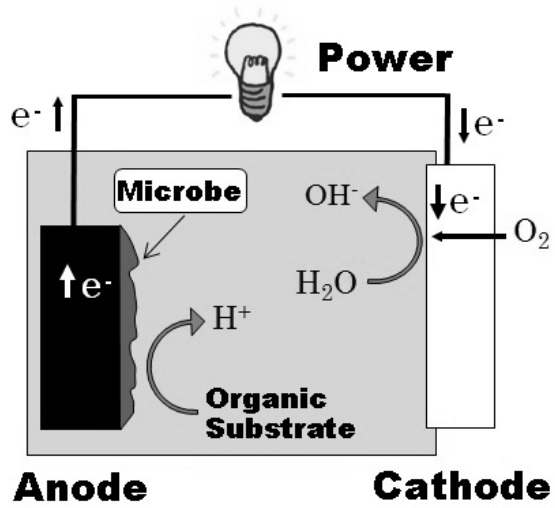
Methane, produced by methanogenic microorganisms, is another potential source of "biofuel" as illustrated in the Figure below.

Methane: an alternative form of energy. Methane gas, released at this Austin, Texas, landfill, is collected and converted into electricity. The structure in the photo is the electrical generator. Methane gas, when used in this way, is an example of a renewable "biofuel." The gas starts to accumulate within months after a landfill is sealed, and gas may be produced at the landfill for 5 years or more.

In many places, methane released from landfills is already being used as a source of inexpensive energy. Another potential source of methane is the large amount of manure, filled with methanogens belonging to Archeae, that is an abundant waste product of animal feedlots.

Microbial fuel cell

Apart from the types discussed earlier, microbial fuel cell is also a type of renewable energy. It is a device that uses microorganisms (mainly bacteria) to convert chemical energy in organisms directly into electrical energy. Its basic working principle is as follows: first, in anaerobic environment of anode chambers, organisms release electrons and protons under microorganic degradation; then, electrons are conveyed through suitable protons media between anode and biological components; furthermore, electrons are delivered to the cathode with outer circuit to form a current. Meanwhile, protons are transported to cathode through proton exchange membrane; finally, an oxidant (typically oxygen) gains electrons at the cathode and combines with protons to form water.



An example of the microbial fuel cell

Chapter (7)

IMPORTANT GROUPS OF BACTERIA

The **procaryotes** (or prokaryotes) consist of millions of genetically- distinct unicellular organisms. What they lack in structural diversity, so well- known among eucaryotes (including the protista), they make up for in their physiological diversity. It is often a particular physiological trait that unifies and distinguishes a particular group of procaryotes to microbiologists. In Bergey's Manualof Determinative bacteriology (1994), the identifiable groups of procaryotes are assembled based on easily-observed phenotypic characteristics such as Gram stain, morphology (rods, cocci, etc), motility, structural features (e.g. spores, filaments, sheaths, appendages, etc.), and on distinguishing physiological features (e.g. anoxygenic photosynthesis, anaerobiasis, methanogenesis, lithotrophy, etc.).

Nowadays, this type of artificial classification scheme has been abandoned in favor of hierarchal taxonomic schemes based on comparative genetic analysis of the nucleotide sequences of the small subunit ribosomal RNA that iscontained in all cellular organisms. In the Second edition of Bergey's Manual of Systematic Bacteriology (2001) as well as the current edition of The Prokaryotes, phylogeny dominates the classification schemes. Such an approach generates the Phylogentic Tree of

Life that lands the procaryotes in two Domains, **Archaea** and **Bacteria.**

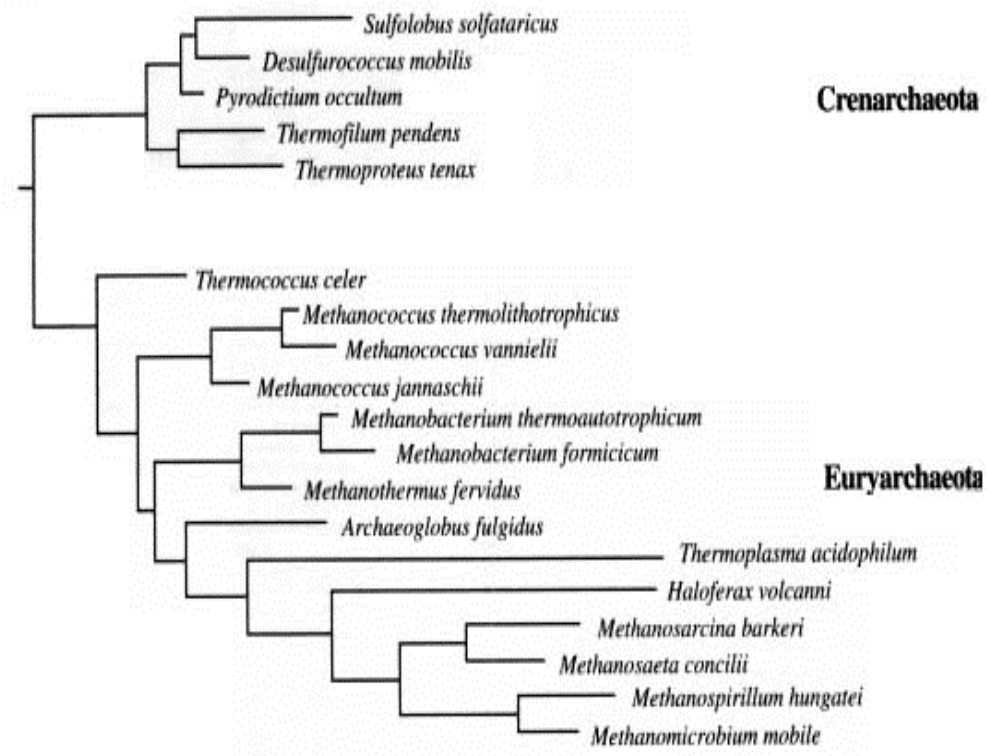
In the ensuing description of procaryotes, groups of organisms are placed under **artificial headings** based on common **structural**, **biochemical** or **ecological** properties. This does not imply close genetic relatedness among different genera in a group. Sometimes all of the members of a group do share a close genetic relatedness; in other cases, members of a group are genetically-unrelated, even to an extent that is greater than exists among all members of the Eucaryotic domain.

ARCHAEA

On the basis of ssrRNA analysis, the Archaea consist of three phylogenetically- distinct groups including **Crenarchaeota**, **Euryarchaeota** and **Korarchaeota**. However, for the Korarchaeota, only their nucleic acids have been detected, and no organisms have been isolated or cultured. Based on their physiology, the Archaea can be organized into three types: **methanogens** (procaryotes that produce methane); **extreme halophiles** (procaryotes that live at very high concentrations of salt (NaCl); and **extreme (hyper) thermophiles** (procaryotes that live at very high temperatures). In addition to the unifying archaeal features that distinguish them from Bacteria (i.e., no murein in cell wall, ether-linked membrane lipids, etc.), the Archaea exhibit other unique structural or biochemical attributes which adapt them to their particular habitats. The **Crenarchaeota**

consists mainly of hyperthermophilic sulfur-dependent procaryotes and the **Euryarchaeota** contains the methanogens and extreme halophiles. ssrRNAs of the **Korarchaeota** have been obtained from hyperthermophilic environments similar to those inhabitated by Crenarchaeota. None of the Korarchaeota have been cultured in the laboratory, although information about them can be inferred from their genome structure.

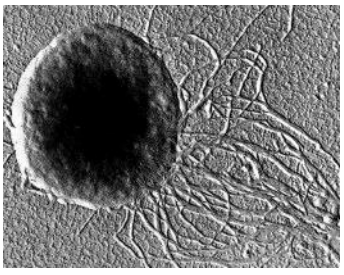
Phylogenetic tree of Archaea



Methanogens

These are obligate anaerobes that will not tolerate even brief exposure to air (O_2). Anaerobic environments are plentiful, however, and include marine and fresh-water sediments, bogs and deep soils, intestinal tracts of animals, and sewage treatment

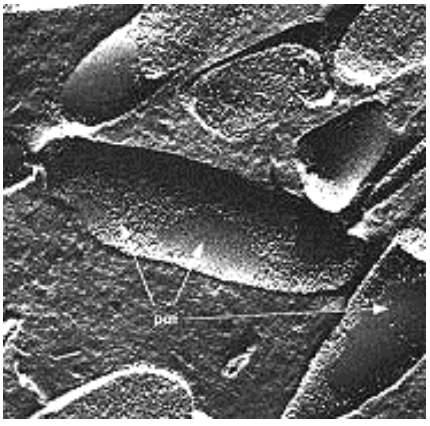
facilities. Methanogens have an incredible type of metabolism that can use H_2 as an energy source and CO_2 as a carbon source for growth. In the process of making cell material from H_2 and CO_2, the methanogens produce methane (CH_4) in a unique energy-generating process. The end product (methane gas) accumulates in their environment. Methanogen metabolism created most the natural gas (fossil fuel) reserves that are tapped as energy sources for domestic or industrial use. Methane is a significant greenhouse gas and is accumulating in the atmosphere at an alarming rate. When rain forests are destroyed and replaced by cows, it is "double-hit" on the greenhouse: (1) less CO_2 is taken up due to removal of the the autotrophic green plants; (2) additional CO_2 and CH_4 are produced as gases by the combined metabolism of the animal and symbiotic methanogens. Methanogens represent a microbial system that can be exploited to produce energy from waste materials. Large amounts of methane are produced during industrial sewage treatment processes, but the gas is usually wasted rather than trapped for recycling.



Methanococcus jannischii. The archaean can be grown in a mineral medium containing only H_2 and CO_2 as sources of energy and carbon for growth within a temperature range of 50 to 86 degrees. Cells are irregular cocci that are motile due to two bundles of polar flagella inserted near the same cellular pole, making it a rare example of a motile coccus.

Extreme halophiles

The organisms live in natural environments such as the Dead Sea, or evaporating ponds of seawater where the salt concentration is very high (as high as 5 molar or 25 percent NaCl). The organisms require salt for growth and will not grow at low salt concentrations (Actually, the cells lyse at low NaCl concentrations). Their cell walls, ribosomes, and enzymes are stabilized by Na^+. *Halobacterium halobium,* the prevalent species in the Great Salt Lake, adapts to the high-salt environment by the development of "purple membrane", formed by patches of light-harvesting pigment in the plasma membrane. The pigment is a type of rhodopsin called **bacteriorhodopsin** which reacts with light in a way that forms a proton gradient on the membrane allowing the synthesis of ATP. This is the only example in nature of non photosynthetic photophosphorylation. The organisms are heterotrophs that normally respire by aerobic means.

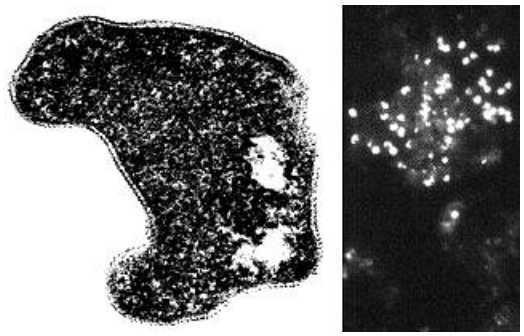


*Halobacterium salinarium*is an extreme halophile that grows at 4 to 5 M NaCl and does not grow below 3 M NaCl. This freeze etched preparation shows the surface structure of the cell membrane and reveals smooth patches of "purple membrane" (bacteriorhodopsin) imbedded in the plasma membrane.

Thermomphiles and extreme thermophiles

They are also called "**hyperthermophiles**" come from several distinct phylogenetic lines of Archaea. These organisms require a very high temperature (80 degrees to 105 degrees) for growth. Their membranes and enzymes are unusually stable at high temperatures. Most of these Archaea require elemental sulfur for growth. Some are anaerobes that use sulfur as an electron acceptor for respiration in place of oxygen. Some are lithotrophs that oxidize sulfur as an energy source. Sulfur-oxidizers grow at low pH (less than pH 2), partly because they acidify their own environment by oxidizing S^o (sulfur) to SO_4 (sulfuric acid). Hyperthermophiles are inhabitants of hot, sulfur-rich environments usually associated with volcanism, such as hot springs, geysers and fumaroles in Yellowstone National Park and elsewhere, and thermal vents ("smokers") and cracks in the ocean floor. *Sulfolobus* was the first hyperthermophilic Archaean discovered by Thomas D. Brock of the University of Wisconsin in 1970. His discovery, along with that of *Thermus aquaticus* (a thermophiloc bacterium) in Yellowstone National Park, launched the field of hyperthermophile biology. (*Thermus aquaticus*is the source of the enzyme **taq polymerase** used in the polymerase chain reaction, PCR., The bacterium has an optimum temperature for growth of 70 degrees.) *Sulfolobus* grows in sulfur-rich, hot acid springs at temperatures as high as 90 degrees and pH values as low as 1. *Thermoplasma,*also discovered by Brock, is a unique

thermophile that is the sole representative of a distinct phylogenetic line of Archaea. *Thermoplasma* resembles the bacterial mycoplasmas in that it lacks a cell wall. *Thermoplasma* grows optimally at 55 degrees and pH 2. Interestingly, it has only been found in self-heating coal refuse piles, which are a man-made waste.



Sulfolobus acidocaldarius: an extreme thermophile that has been found in geothermally-heated acid springs, mud pots and surface soils with temperatures from 60 to 95 degrees C, and a pH of 1 to 5. Left: Electron micrograph of a thin section (85,000X). Under the electron microscope the organism appears as irregular spheres which are often lobed. Right: Fluorescent photomicrograph of cells attached to a sulfur crystal. Fimbrial-like appendages have been observed on the cells attached to solid surfaces such as sulfur crystals.

Although the Archaea are often inhabitants of unusual or extreme environments, there may be corresponding species of Bacteria, and even eucaryotes, in these habitats as well. No bacterium can produce methane, but in many anaerobic environments Bacteria are found in association with methanogens. With regard to acid tolerance, a bacterium, *Thiobacillus*, has been observed growing at pH near 0. A eucaryotic alga, *Cyanidium*, has also been found growing near pH 0. In superheated environments (greater than 100 degrees), Archaea may have an exclusive hold, but Bacteria have been isolated from boiling hot springs in Yellowstone National Park and other parts of the world. No bacterium grows

at the highest salt concentration which supports the growth of the halobacteria, but osmophilic yeasts and fungi can grow at correspondingly low water actvities where sugar is the solute in high concentration.

BACTERIA

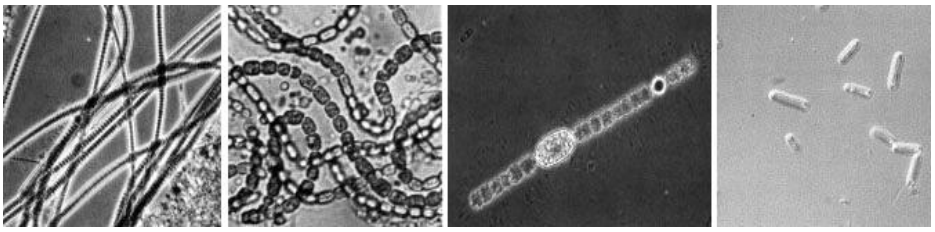
Phylogenetic analysis of the Bacteria has demonstrated the existence of at least 13 distinct groups (Figure 5), but many groups consist of members that are phenotypically and physiologically unrelated, and sometimes phylogenetically unrelated. The current edition of Bergey's Manual of Systematic Bacteriology (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.

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



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.

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. The are functionally analogous to bacterial endospores, but they bear little

resemblance and lack the extraordinary resistance properties of endospores.

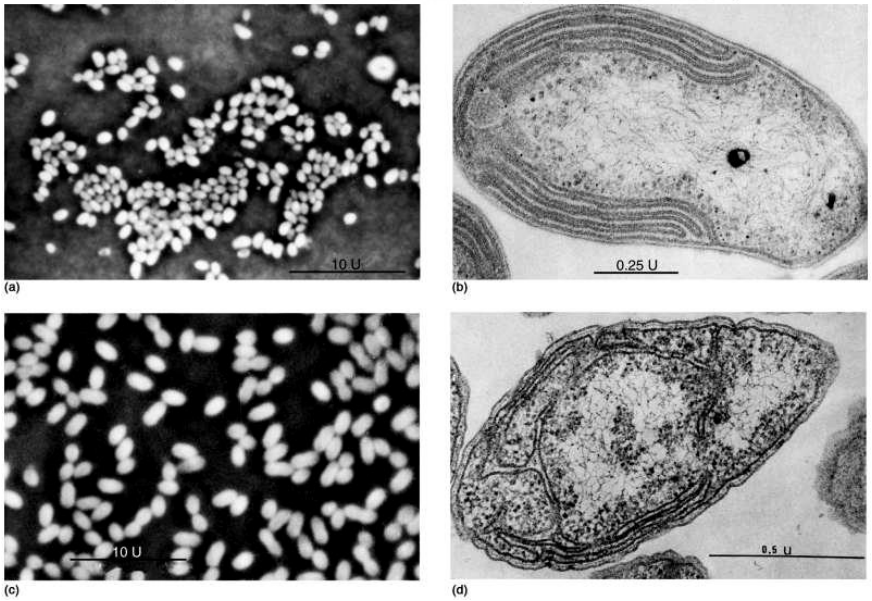
A few cyanobacteria are symbionts of liverworts, ferns, cycads, flagellated protozoa, and algae, sometimes occurring as endosymbionts of the eucaryotic cells. In the case of the water fern, *Azolla*, the cyanobacterial endophyte (a species of *Anabaena)* fixes nitrogen that becomes available to the plant.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

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.

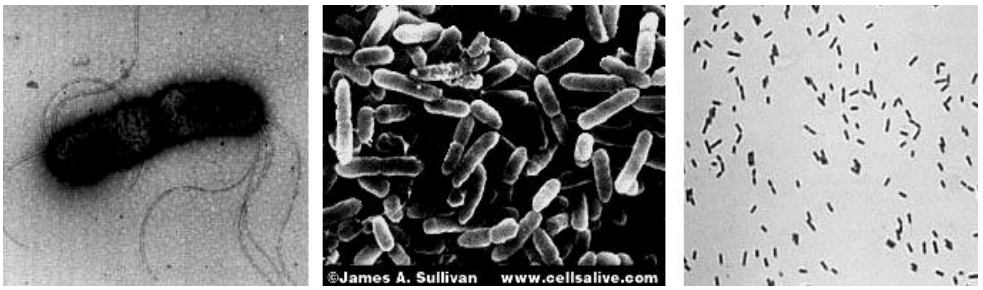


(a), (b) *Nitrobacter winogradskyi* and (c), (d) *Nitrosomonas europaea*

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.



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. Pseudomonas species are discussed elsewhere in the text at Opportunistic Infections caused by *Pseudomonas aeruginosa* and The Genus *Pseudomonas*.

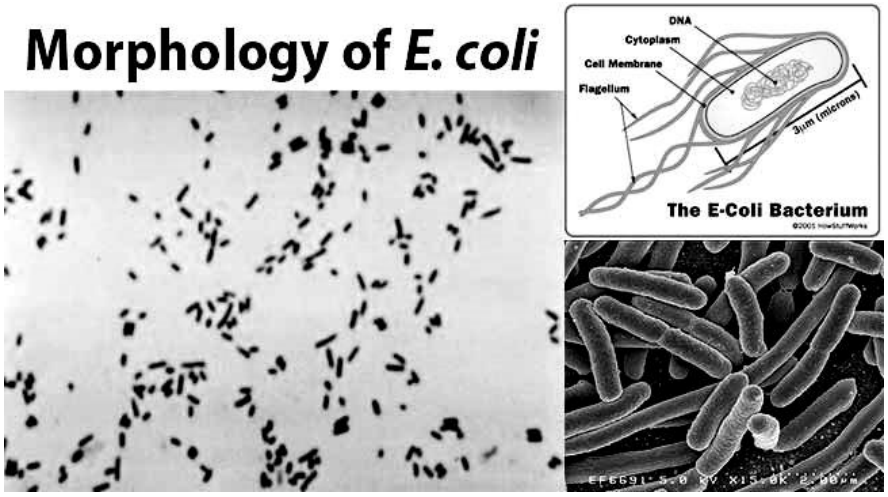
Among some interesting or important ecologic relatives of the pseudomonads are *Rhizobium*and *Bradyrhizobium*, species that fix nitrogen in association with leguminous plants, and related *Agrobacterium* species that cause tumors ("galls") in plants. These bacteria are discussed later in this article 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.

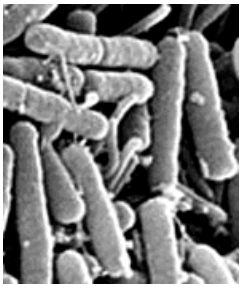
Escherichia coli is, of course, the type species of the enterics. *E. coli* is such a regular inhabitant of the intestine of humans that it is used by public health authorities as an indicator of fecal pollution of drinking water supplies, swimming beaches, foods, etc. *E. coli* is the most studied of 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.**



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

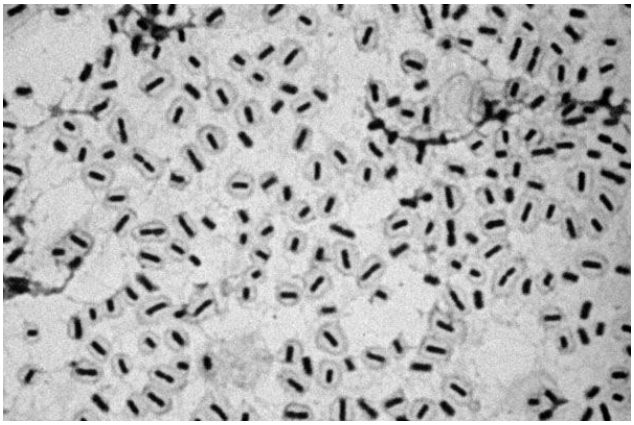
causes **bubonic plague**, and *Erwinia,* an important pathogen of plants.



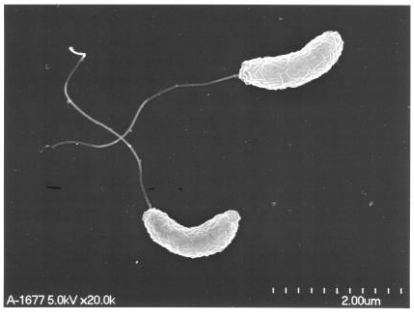
Erwinia carotovora

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.



Haemophilus influenzae



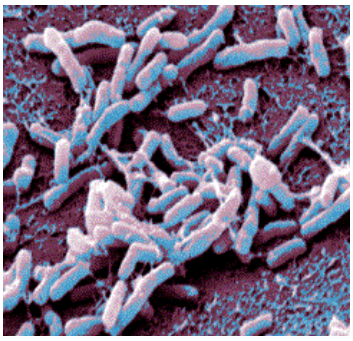
Vibrio cholerae

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.

Bean root nodules



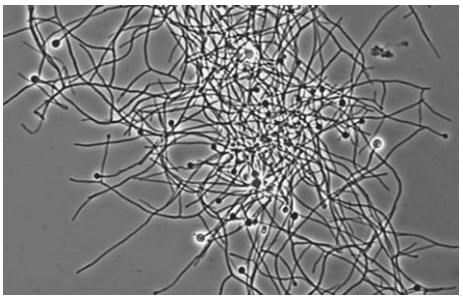
Rhizobium

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.

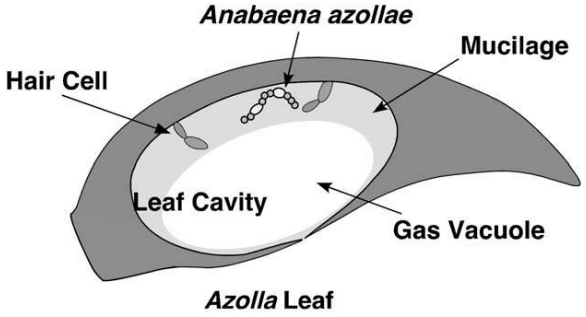
In this symbiosis the bacterium invades the root of the plant and fixes nitrogen which it shares with the plant. The plant provides a favorable habitat for the bacterium and supplies it with nutrients and energy for efficient nitrogen fixation. *Rhizobium* and *Bradyrhizobium* are Gram-negative aerobes 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.



Frankia showing hyphae and vesicles (site for nitrogenase)

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 (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. A related process call **dissimilatory nitrate reduction**, conducted by certain *Bacillus* species, reduces

NO_3 to NH_3. One rationale for tilling the soil is to keep it aerobic, in order to discourage these facultative processes in *Pseudomonas* and *Bacillus*, which are ubiquitous inhabitants.

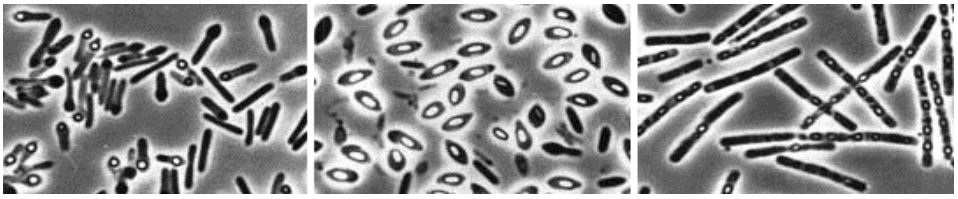
Lactic acid bacteria

These 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

They 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. Some spore formers 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 increasingly recognized as an agent of food poisoning. *Clostridium botulinum* causes **botulism** a form of food- poisoning, and *Clostridium tetani* causes **tetanus**.



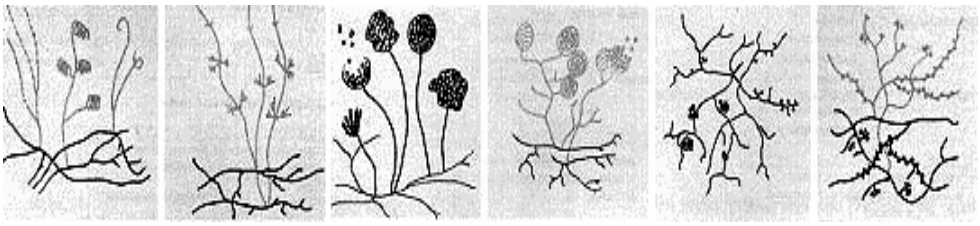
Endospore-forming bacilli (phase contrast illumination). Endospores are dehydrated, refractile cells appearing as points of bright light under phase microcsopy. Endospore-forming bacteria are characterized by the location (position) of the endospore in the mother cell (sporangium) before its release.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

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.

Actinomycetes and related bacteria

They are a large group of Gram-positive bacteria that usually grow by filament formation, or at least show a tendency towards branching and filament formation. Many of the organisms can form resting structures called spores, but they are not the same as endospores. Branched forms superficially resemble molds and are a striking example of convergent evolution of a procaryote and a eucaryote together in the soil habitat. Actinomycetes such as *Streptomyces* have a world-wide distribution in soils. They are important in aerobic decomposition of organic compounds and have an important role in biodegradation and the carbon cycle. Products of their metabolism, called **geosmins**, impart a characteristic earthy odor to soils. Actinomycetes are the main producers of antibiotics in industrial settings, being the source of most tetracyclines, macrolides (e.g. erythromycin), and aminoglycosides (e.g. streptomycin, gentamicin, etc.). Two bacteria in this diverse group are important pathogens of humans: *Mycobacterium tuberculosis* is the cause of **tuberculosis**; *Corynebacterium diphtheriae* is the cause of **diphtheria**.



Schematic diagrams illustrating mycelial growth and spore formation in several genera of actinomycetes.

Plant-pathogenic bacteria

Many economically-important diseases of plants are caused by members of the Bacteria. It is estimated that one-eighth of the crops worldwide are lost to diseases caused by bacteria, fungi or insects. Almost all kinds of plants can be affected by bacterial diseases, and many of these diseases can be extremely destructive.

Almost all plant-pathogenic bacteria are Gram-negative bacilli, usually affiliated with the pseudomonads or enterics (above). The symptoms of bacterial disease in plants are described by a number of terms such as spots, blights, soft rots, wilts, and galls. Bacterial spots of various sizes on stems, leaves, flowers and fruits are usually caused by *Pseudomonas* or *Xanthomonas* species. Bacteria may cause **spots** by producing toxins that kill cells at the site of infection. **Blights** are caused by rapidly developing necrosis (dead, discolored areas) on stems, leaves and flowers. Fire blight in apples and pears, caused by *Erwinia amylovora*, can kill young trees within a single season. Bacterial **soft rots** occur most commonly in fleshy vegetables such as potatoes or onions or fleshy fruits such as tomatoes and eggplants. The most destructive soft rots are caused by *Erwinia* species that attack fruits and vegetables at the post-harvest stage.

Bacterial vascular **wilts** mainly affect herbaceous plants. The bacteria invade the vessels of the xylem, where they multiply, interfering with the movement of water and inorganic nutrients

and resulting in the wilting and the death of the plants. The bacteria commonly degrade portions of the vessel walls and can even cause the vessels to rupture. Once the walls have ruptured, the bacteria then spread to the adjacent parenchyma tissues, where they continue to multiply. In some bacterial wilts, the bacteria ooze to the surface of the stems or leaves through cracks formed over cavities filled with cellular debris, gums, and bacteria. More commonly, however, the bacteria do not reach the surface of the plant until the plant has been killed by the disease. Wilts of alfalfa and bean plants are cause by species of *Clavibacter;* bacterial wilt of cucurbits, such as squashes and watermelons, are cause by *Erwinia tracheiphila*; the black rot of crucifers such as cabbage is caused by *Xanthomonas campestris*. The most economically-important wilt of plants is caused by *Pseudomonas solanacearum* which affects 44 genera of plants, including such major crops as bananas, peanuts, tomatoes, potatoes, eggplants and tobacco. This disease occurs worldwide in tropical, subtropical, and warm temperate areas.

Mycoplasmas (discussed above) have been identified in more than 200 plant species and associated with more than 50 plant diseases, many with symptoms of yellowing. Among these plant-pathogens are the spiroplasmas (genus *Spiroplasma*), which are pleomorphic, ovoid or spiral-shaped cells which are motile by means of a rotary or screw-like motion. Intracellular fibrils are thought to be responsible for their movement. The organisms

have been isolated from the fluids of vascular plants and from the gut of insects that feed on these fluids. Some have been cultured on artificial media, including *Spiroplasma citri*, which is isolated from the leaves of citrus plants, where it causes citrus stubborn disease, and from corn plants suffering from corn stunt disease. A number of other mycoplasma-like organisms (sometimes called **MLOs**) have been detected in diseased plants by electron microscopy, which has been taken as evidence that these organisms may be more involved in plant disease than previously realized.

The causative agent of a common plant disease, termed **crown gall**, is *Agrobacterium tumefaciens*. The disease is characterized by large **galls** or swellings that form on the plant at the site of infection, usually near the soil line. Crown gall is a problem in nurseries, affecting ornamental plants and fruit stock, and it may be a serious disease in grapes. Because of their role in the genetic engineering of plants, the molecular biology of these bacteria is intensively studied.

***Agrobacterium* galls on plant stem**

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South **V**alley **U**niversity

Faculty of **S**cience Time allowed: 3 hours.
Botany **D**epartment Applied Microbiol. Diploma

Bacteriology & Virology exam.

Part (I): Bacteriology

Answer two only of the following:

(1) a- Discuss the different approaches for measuring bacterial growth giving examples on each.

b) How the bacterial cell transports the nutrients from the surrounding environment. Give an idea about different transporting systems and their energy requirements.

(2) a) Explain, with drawing if possible, the structure and function of both the bacterial cell wall and plasma membrane.

b) What are the main agents used for killing or stopping bacterial growth?

(3) a) What are the main appendages attached to the bacterial cell and what are their functions.

b) Give short notes on the ideal growth curve of bacteria and its growth phases.

Part (II): Virology

Answer the following questions:-

(1): Write on each of the following:

(A) Pathogenesis & **T**ransmission of **M**easles **&H**epatitis **A** viruses.

(B) How **V**iruses Cause **D**isease.

(2)

(A) Compare between the **R**eplication of RNA and DNA **V**iruses.

(B) What is meant by the following **T**erms: **O**ncogenic **V**iruses, **P**rimary **R**eplication, **T**issue/**C**ell tropism, **P**rimary & **S**econdary **V**iremia,

South **V**alley **U**niversity

Faculty of **S**cience Time allowed: 3 hours.

Botany **D**epartment Applied Microbiol. Diploma

Bacteriology & Virology exam.

Part (I): Bacteriology

Answer two only of the following:

(1) a- Discuss two different approaches for measuring bacterial growth giving examples on each.

b) How the bacterial cell moves in the surrounding environment? What is the tactic behaviour? How to detect bacterial motility?.

(2) a) Explain, with drawing if possible, the structure and functions of the bacterial cell wall.

b) What are the main factors that affect bacterial growth? Discuss two of these factors and divide bacterial groups on the bases of oxygen and temperature requirements

(3) a) What are the differences between bacteriostatic and bactericidal agents- antiseptics and disinfectants? Give examples on each.

b) Give short notes on the use of bacteria in food and medicine.

South Valley University
Faculty of Science
Department of Botany

Bacteriology Exam
Applied Microbiol. Diploma.
Time: 2 hrs

2020

Part I: Mark on the correct answer as false (F) or True (T).

1) DNA of the bacterial cell is tightly enclosed within a thin membrane.

2) The bacterial biofilm is mainly composed of slime.

3) Flagella can be stained by the negative staining method.

4) Auxotrophs are those bacteria that do not require growth factors.

5) Bacterial surface structure is a permeability barrier for certain molecules.

6) Murein strands are connected together by interpeptidation.

7) The outer membrane of Gram -ve bacteria contains some rare sugars.

8) Spheroplasts are thick- walled forms of bacteria that survive antibiotic therapy.

9) Endospore formation is a kind of reproduction in bacteria.

10) Growing bacteria in the laboratory requires the addition of buffers to culture media.

Part II: Choose the correct answer:

1) Bacterial growth factors are those:

A. Synthesized by few bacterial species.
B. Not synthesized by some bacteria.
C. Synthesized by some bacteria for the benefit of others.
D. Chemical reagents added to the culture media.

2) Synthetic culture medium is that:

A. Containing organic carbon sources.
B. Composed of undefined chemical composition.
C. Containing complex natural materials.
D. Its chemical composition is exactly known.

3) Osmophilic bacteria are usually those:

A. Intolerant to osmotic pressure.
B. Live in neutral pH conditions.
C. Live in distilled water.
D. Live in high sugar concentrations.

4) Counting CFU's in a plate medium is a method called:

A. Direct cell number count.
B. Indirect cell number count.
C. Indirect cell mass count.
D. Direct cell mass count.

5) In the exponential growth phase, the generation time is:

A. The longest.
B. Undefined.
C. Suboptimal.
D. The shortest.

6) Growth synchronization can be achieved by:

A. Using selective medium.
B. Physical, mechanical and chemical methods.
C. Using buffers.
D. Heating to 60º C.

7) The backbone of murein is composed of:

A. Different sugars and lipids.
B. Special protein molecules.
C. Alternating sugar molecules connected by peptides.
D. DNA and RNA molecules.

8) Bacterial vertical evolution is:

A. A sexual reproduction method.
B. Changes due to antibiotic therapy.
C. Natural mutation and selection.
D. Due to attack by bacteriophages.

9) Fimbriae are:

A. One of the molecule porters in the transport systems.
B. Short hair- like proteins on cell surface.
C. Structures that help in motility.
D. Molecules involved in protein synthesis.

10) Chemotaxis is:

A. The ability of bacteria to degrade chemicals.
B. Chemicals that enter bacterial cell through membrane.
C. Carriers of chemicals.
D. Response of bacteria towards chemicals.