

Lab 1+2: Introduction of medical Laboratory

Bacteriology: is branch of microbiology dealing with study of bacteria, their structure, organization habitat, cultivation identification differentiation, pathogenicity, association, and with their application in medicine m agriculture, industry, and biotechnology, and importance in our daily life style, it used for Preventing spoilage (mainly food), Medical importance, Antibiotics, Bio-remediation, Prebiotics and Probiotics.

Bacteria: are prokaryotic, single (unicellular) called microscopic microorganisms, They are found practically everywhere on Earth and live in some of the most unusual and seemingly inhospitable places, which can exist either as independent (free-living) organisms or as parasites (dependent upon another organism for life). Evidence shows that bacteria were in existence as long as 3.5 billion years ago, making them one of the oldest living organisms on the Earth.

Antoni van Leeuwenhoek became the first to study bacteria under the microscope. During the nineteenth century, the French scientist Louis Pasteur and the German physician Robert Koch demonstrated the role of bacteria as pathogens (causing disease). The twentieth century saw numerous advances in bacteriology, indicating their diversity, ancient lineage, and general importance. a number of scientists around the world made contributions to the field of microbial ecology, showing that bacteria were essential to food webs and for the overall health of the Earth's ecosystems. The discovery that some bacteria produced compounds lethal to other bacteria led to the development of antibiotics, which revolutionized the field of medicine.

Bacteria have very complex structure despite their small size. Even though bacteria are single-celled organisms, which lack chlorophyll pigments. The cell structure is simpler than that of other organisms as there is no nucleus or membrane bound organelles. Due to the presence of a rigid cell wall, bacteria maintain a definite shape, though they vary as

shape, size and structure. The metric unit micrometres ($1/1,000,000$ or 10^{-6} of a meter) are used to measure bacterial size.

The small size of microbes and the consequent need to deal with cultures that contain many millions of microbial cells require special procedures for their safe use. Activities involving micro-organisms are controlled by the Control of Substances Hazardous to Health (COSHH).

❖ General Laboratory Directions

1. Always read the assigned laboratory material *before* the start of the laboratory period.
2. Before entering the laboratory, remove coats, jackets, and other outerwear. These should be left outside the laboratory, together with any backpacks, books, papers, or other items not needed for the work.
3. To be admitted to the laboratory, each student should wear a fresh, clean, knee-length laboratory coat.
4. At the start and end of each laboratory session, students should clean their assigned bench-top area with a disinfectant solution provided. That space should then be kept neat, clean, and uncluttered throughout each laboratory period.
5. Learn good personal habits from the beginning: Tie back long hair neatly, away from the shoulders. **Do not** wear jewellery to laboratory sessions. Keep fingers, pencils, and such objects out of your mouth.
Do not smoke, eat, or drink in the laboratory. **Do not** lick labels with your tongue. Use tap water or preferably, self-sticking labels. **Do not** wander about the laboratory. Unnecessary activity can cause accidents, distract others, and promote contamination.
6. Each student will need matches, bibulous paper, lens paper, a china-marking pencil, and a 100-mm ruler (purchased or provided). A black, waterproof marking pen may be used to mark petri plates and tubes.
7. Keep a complete record of all your experiments, your completed work can be removed from the manual and submitted to the instructor for evaluation.

8. Discard all cultures and used glassware into the container labelled *Contaminated*. (This container will later be sterilized.) Plastic or other Disposable items should be discarded separately from glassware in containers to be sterilized.

- *Never place contaminated pipettes on the bench top.*
- *Never discard contaminated cultures, glassware, pipettes, tubes, or slides in the wastepaper basket or garbage can.*
- *Never discard contaminated liquids or liquid cultures in the sink.*

9. If you are in doubt as to the correct procedure, double-check the manual. If doubt continues, consult your instructor. Avoid asking your neighbour for procedural help.

10. If you should spill or drop a culture or if any type of accident occurs, *call the instructor immediately*. Place a paper towel over any spill and pour disinfectant over the towel. Let the disinfectant stand for 15 minutes, and then clean the spill with fresh paper towels. Remember to discard the paper towels in the proper receptacle and wash your hands carefully.

11. Report any injury to your hands to the instructor either before the laboratory session begins or during the session.

12. **Never** remove specimens, cultures, or equipment from the laboratory under any circumstances.

13. Before leaving the laboratory, carefully wash and disinfect your hands. Arrange to launder your lab coat so that it will be fresh for the next session.

14. Hands and bench tops are kept clean with disinfectants, laboratory coats are worn, long hair is tied back, and working areas are kept clear of all unnecessary items. Containers used for specimen collection or culture material are pre-sterilized and capped to prevent entry by unsterile air, and sterile tools are used for transferring specimens or cultures. *Nothing* is placed in the mouth.

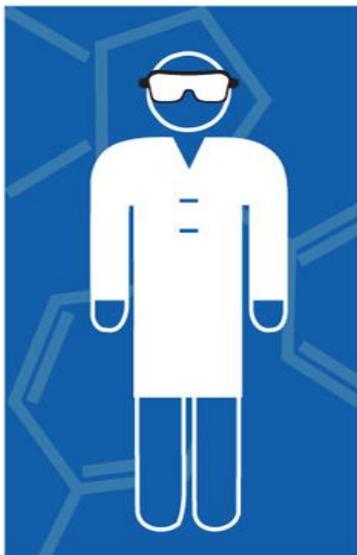
15. In general, all safety procedures and precautions followed in the microbiology laboratory are designed to:

- *Restrict microorganisms present in specimens or cultures* to the containers in which they are collected, grown, or studied.
- *Prevent environmental microorganisms* (normally present on hands, hair, clothing, laboratory benches, or in the air) from entering specimens or cultures and interfering with results of studies.

Planning ahead is essential when embarking on practical bacteriology investigations.

There are five areas for Consideration.

- Preparation and sterilization of equipment and culture media.
- Preparation of microbial cultures as stock culture for future investigations and inoculum for the current Investigation.
- Inoculation of the media with the prepared culture.
- Incubation of cultures and sampling during growth.
- Sterilisation and safe disposal of all cultures and decontamination of all contaminated equipment (See appendix 1, 2).



Lab Safety Rules

Science labs offer great opportunities for learning, teaching, and research. They also pose hazards that require proper safety precautions.



Stay safe when conducting your labs by following these guidelines.



Dress appropriately

Tie back long hair, and wear suitable gloves, goggles, and other protective equipment.

Proper supervision

Don't perform lab experiments without instructor supervision (unless given permission to do so).



Know location of emergency numbers & safety equipment

Know the location of safety equipment and emergency phone numbers (such as poison control) so you can access them quickly if necessary.



No food

Don't eat or drink in the lab—and never taste chemicals.



ID hazards

Identify hazardous materials before beginning labs.



Be attentive

Be attentive while in the lab. Don't leave lit Bunsen burners unattended or leave an experiment in progress.

Be careful when handling hot glassware

Turn off all heating appliances when not in use. Keep flammable objects away from your workspace.



Keep a clean workspace

Don't obstruct work areas, floors, or exits. Keep coats, bags, and other personal items stored in designated areas away from the lab. Don't block sink drains with debris.



Handle glassware carefully

Properly dispose of anything that breaks. Report cuts, spills, and broken glass to your instructor immediately.



Clean up

After completing the lab, carefully clean your workspace and the equipment, and wash your hands.

Sources: Carolina Biological Supply Company. "Lab Safety Dos and Don'ts for Students." <http://www.carolina.com/teacher-resources/Interactive/lab-safety-instructions/tr11076.tr>

❖ General size and forms of bacteria

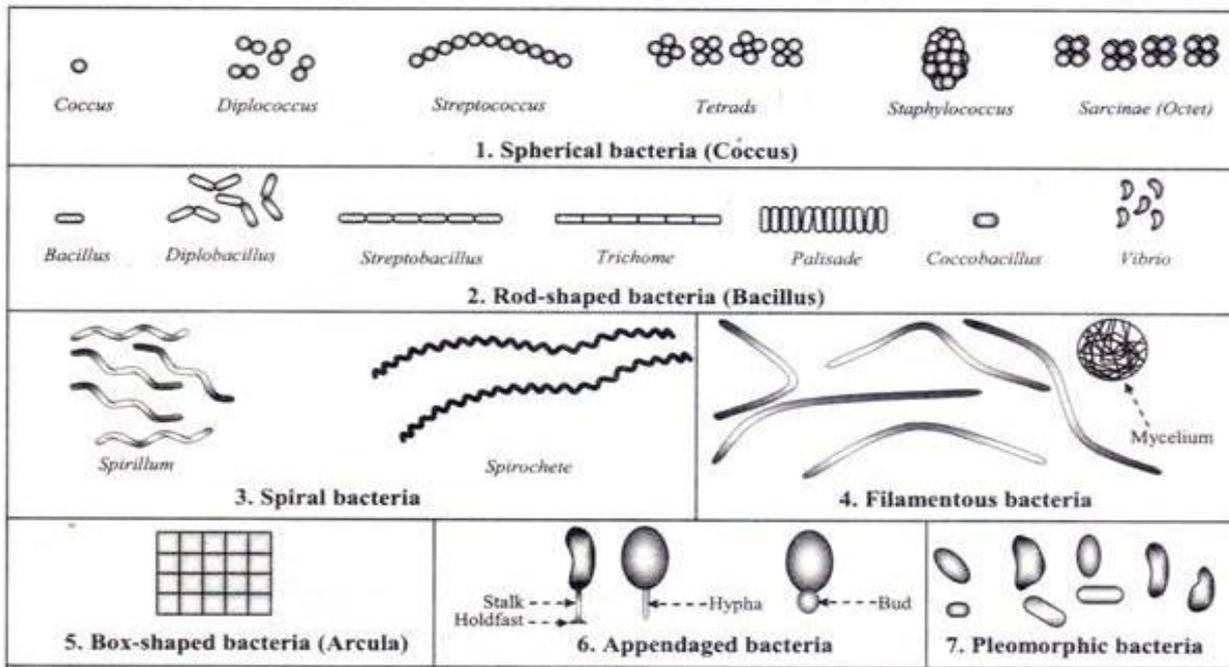


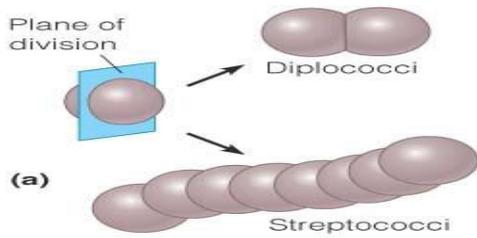
Figure 1: general forms of bacteria

When viewed under light microscope, most bacteria appear in variations of three major shapes: the rod (bacillus), the sphere (coccus) and the spiral type (vibrio). In fact, structure of bacteria has two aspects, arrangement and shape. So far as the **arrangement** is concerned, it may **pair** (diplo), **Grape-like clusters** (staphylo) or **Chains** (strepto). **In shape** they may principally be **Rods** (bacilli), **Spheres** (cocci), and **Spirals** (spiralled). However pleomorphic bacteria can assume several shapes.

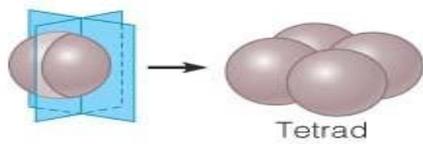
There are exceptions to the three basic shapes of coccus, bacillus, and spiral. They include sheathed, stalked, **filamentous**, square, star-shaped, spindle-shaped, lobed, **trichome-forming**, and pleomorphic bacteria.

1. Coccus arrangement

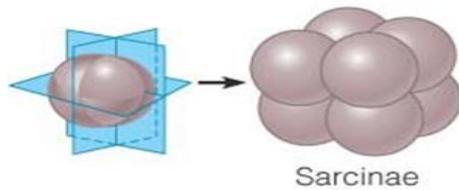
A. Division in one plane produces either a diplococcus or streptococcus arrangement. Examples of diplococcus: *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Neisseria gonorrhoeae*, etc. Example of cocci arranged in chains *Streptococcus pyogenes*, *Streptococcus agalactiae*



B. Division in two planes produces a tetrad arrangement. i.e. arranged in squares of 4 ex. (*Micrococcus luteus*, *Tetragenococcus*)



C. Division in three planes produces a Sarcina arrangement. i.e. in arranged cubes of 8 d. Ex. : *Sarcina ventriculi*, *Sarcina ureae*, etc.



D. Division in random planes produces a staphylococcus arrangement, staphylococcus: cocci arranged in irregular, often grape-like clusters ex. *Staphylococcus aureus*,

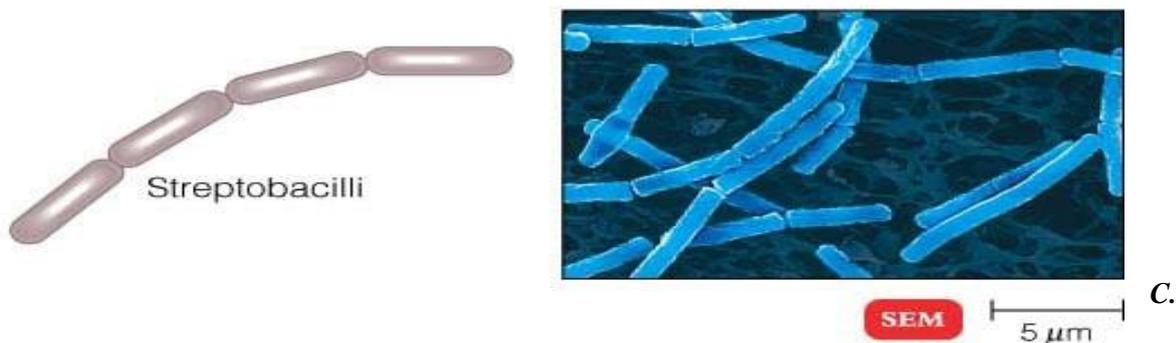


2. Rod or bacillus arrangement

A. bacillus: single bacilli ex. (*Escherichia coli*) or baired, Examples of Diplobacilli: *Coxiella burnetii*, *Moraxella bovis*, *Klebsiella rhinoscleromatis*, etc.



B. streptobacillus: bacilli arranged in chains as the cells divide in one plane, Ex.: *Streptobacillus moniliformis*

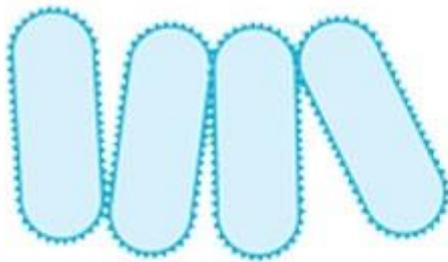


Coccobacillus: oval and similar to a coccus, i.e. These are so short and stumpy that they appear ovoid. They look like coccus and bacillus.



D. Palisades: The bacilli bend at the points of division following the cell divisions, resulting in a palisade arrangement resembling a picket fence and angular patterns that look like Chinese letters.

Ex.: *Corynebacterium diphtheriae*



Palisades



3. Spirilla arrangement

A. vibrio a curved or comma-shaped rod, They are comma-shaped bacteria with less than one complete turn or twist in the cell. E.g. (*Vibrio cholerae*)



Vibrio



SEM

2 μ m

B. Spirillum They have a thick, rigid spiral structure. Spirillum with many turns can superficially resemble spirochetes. They do not have outer sheath and endoflagella, but have typical bacterial flagella.

Ex.: *Campylobacter jejuni*, *Helicobacter pylori*, *Spirillum winogradskyi*, etc.



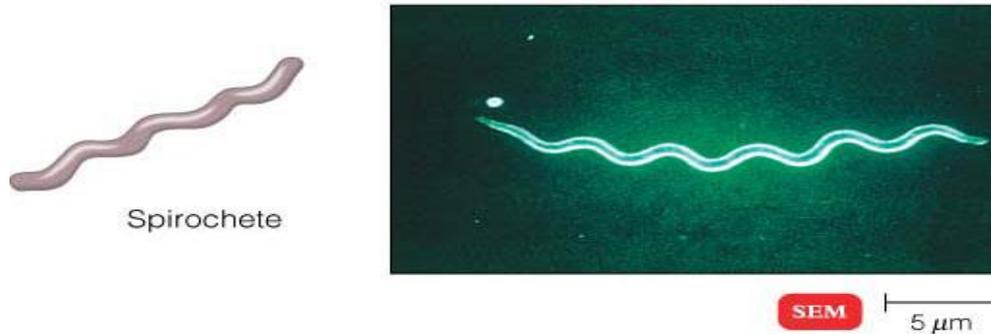
Spirillum



SEM

2 μ m

C. Spirochete c. spirochete: Thin, flexible spirals Spirochetes have a helical shape and flexible bodies. Spirochetes move by means of axial filaments, which look like flagella contained beneath a flexible external sheath but lack typical bacterial flagella. Ex.: *Leptospira* species (*Leptospira interrogans*, *Treponema pallidum*, *Borrelia recurrentis*, etc.



❖ Exceptions to the above shapes

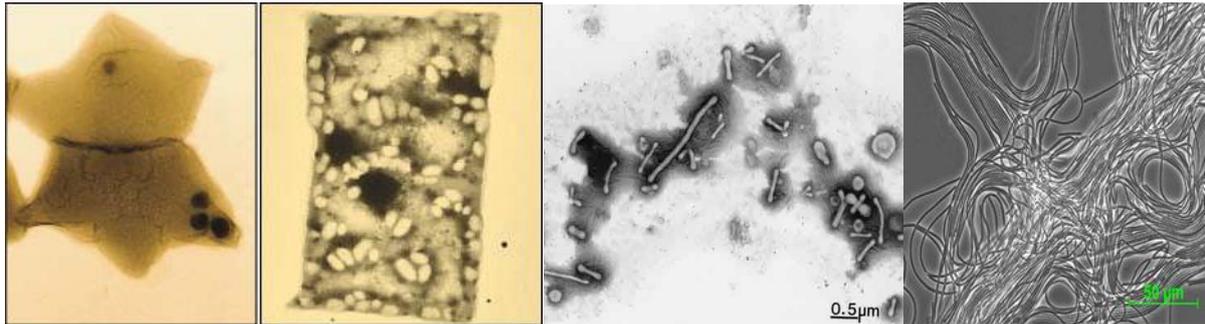
There are exceptions to the three basic shapes of coccus, bacillus, and spiral. They include sheathed, stalked, filamentous, square, star-shaped, spindle-shaped, lobed, trichome-forming, and pleomorphic bacteria.

1. Filamentous Bacteria: They are very long thin filament-shaped bacteria. Some of them form branching filaments resulting in a network of filaments called 'mycelium'. Ex.: *Candidatus Savagella*, fig a

2. Star Shaped Bacteria: ex.: *Stella* fig b

3. Rectangular Bacteria: Ex.: *Haloarcula* spp. (*H. vallismortis*, *H. marismortui*) fig c

4. Pleomorphic Bacteria: These bacteria do not have any characteristic shape unlike all others described above. They can change their shape. In pure cultures, they can be observed to have different shapes. Fig d, Ex.: *Mycoplasma pneumoniae*, *M. genitalium*, etc.



-a-

-b-

-c-

-d-

Fig 2: Other bacterial shapes

❖ Bacterial cell structure

Bacteria are prokaryotes, lacking well-defined nuclei and membrane-bound organelles, and with chromosomes composed of a single closed DNA circle.

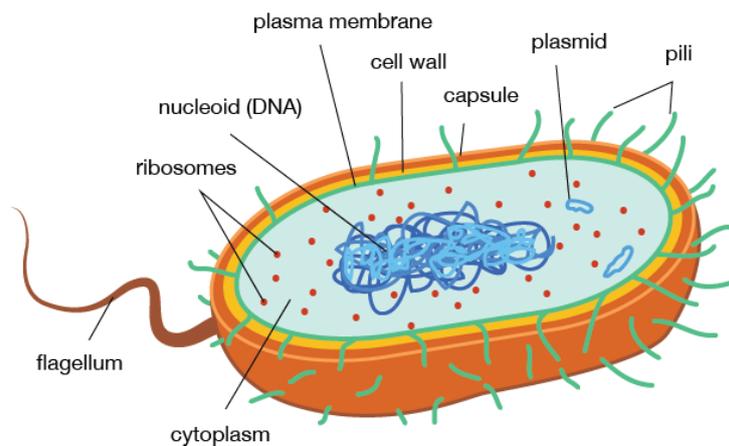


Fig 3: bacterial cell structure

- **Capsule** - Some species of bacteria have a third protective covering, a capsule made up of polysaccharides (complex carbohydrates). Capsules play a number of roles, but the most important are to keep the bacterium from drying out and to protect it from phagocytosis (engulfing) by larger microorganisms. The capsule is a major virulence factor in the major disease-causing bacteria, such as *Escherichia coli* and *Streptococcus*

pneumoniae. Non-encapsulated mutants of these organisms are a virulent, i.e. they don't cause disease.

- **Cell Envelope** - The cell envelope is made up of two to three layers: the interior cytoplasmic membrane, the cell wall, and -- in some species of bacteria -- an outer capsule.
- **Cell Wall** - Each bacterium is enclosed by a rigid cell wall composed of peptidoglycan, a protein-sugar (polysaccharide) molecule. The wall gives the cell its shape and surrounds the cytoplasmic membrane, protecting it from the environment. It also helps to anchor appendages like the pili and flagella; the strength of the wall is responsible for keeping the cell from bursting when there are large differences in osmotic pressure between the cytoplasm and the environment.

Cell wall composition varies widely amongst bacteria and is one of the most important factors in bacterial species analysis and differentiation. For example, a relatively thick, structure that makes it possible to distinguish two basic types of bacteria. A technique devised by Danish physician Hans Christian Gram in 1884, uses a staining to differentiate between the two forms. When exposed to a gram stain, gram-positive bacteria retain the purple colour of the stain because the structure of their cell walls traps the dye. In gram-negative bacteria, the cell wall is thin and releases the dye readily when washed with an alcohol or acetone solution.

- **Cytoplasm** - The cytoplasm, or protoplasm, of bacterial cells is where the functions for cell growth, metabolism, and replication are carried out. It is a gel-like matrix composed of water, enzymes, nutrients, wastes, and gases and contains cell structures such as ribosomes, a chromosome, and plasmids. The cell envelope encases the cytoplasm and all its components. Unlike the eukaryotic (true) cells, bacteria do not have a membrane enclosed nucleus. The chromosome, a single, continuous strand of

DNA, is localized, but not contained, in a region of the cell called the nucleoid. All the other cellular components are scattered throughout the cytoplasm.

- **Cytoplasmic Membrane** - A layer of phospholipids and proteins, called the cytoplasmic membrane, encloses the interior of the bacterium, regulating the flow of materials in and out of the cell. This is a structural trait bacteria share with all other living cells; a barrier that allows them to selectively interact with their environment. Membranes are highly organized and asymmetric having two sides, each side with a different surface and different functions.

- **Ribosomes** - Ribosomes are microscopic "factories" found in all cells, including bacteria. They translate the genetic code from the molecular language of nucleic acid to that of amino acids—the building blocks of proteins. Proteins are the molecules that perform all the functions of cells and living organisms. Bacterial ribosomes are similar to those of eukaryotes, but are smaller and have a slightly different composition and molecular structure. Bacterial ribosomes are never bound to other organelles as they sometimes are (bound to the endoplasmic reticulum) in eukaryotes, but are free-standing structures distributed throughout the cytoplasm. There are sufficient differences between bacterial ribosomes and eukaryotic ribosomes that some antibiotics will inhibit the functioning of bacterial ribosomes, but not a eukaryote's, thus killing bacteria but not the eukaryotic organisms they are infecting.

- **Nucleoid** - The nucleoid is a region of cytoplasm where the chromosomal DNA is located. It is not a membrane bound nucleus, but simply an area of the cytoplasm where the strands of DNA are found. Most bacteria have a single, circular chromosome that is responsible for replication, although a few species do have two or more. Smaller circular auxiliary DNA strands, called plasmids, are also found in the cytoplasm.

- **Plasmids** – are small, extra-chromosomal genetic structures carried by many strains of bacteria. Like the chromosome, plasmids are made of a circular piece of DNA.

Unlike the chromosome, they are not involved in reproduction. Only the chromosome has the genetic instructions for initiating and carrying out cell division, or binary fission. Plasmids are passed on to other bacteria through two means. For most plasmid types, copies in the cytoplasm are passed on to daughter cells during binary fission. Plasmids have been shown to be instrumental in the transmission of special properties, such as antibiotic drug resistance, resistance to heavy metals, and virulence factors necessary for infection of animal or plant hosts. The ability to insert specific genes into plasmids has made them extremely useful tools in the fields of molecular biology and genetics, specifically in the area of genetic engineering.

- **Flagella** - Flagella (singular, flagellum) are hair like structures that provide a means of locomotion for those bacteria that have them. They can be found at either or both ends of a bacterium or all over its surface. The flagella help the bacterium move toward nutrients; away from toxic chemicals; or, in the case of the photosynthetic cyanobacteria; toward the light.
- **Pili** - Many species of bacteria have pili (singular, pilus), small hair like projections emerging from the outside cell surface. These assist the bacteria in attaching to other cells and surfaces, such as teeth, intestines, and rocks. Without pili, many disease-causing bacteria lose their ability to infect because they're unable to attach to host tissue. Specialized pili are used for conjugation, during which two bacteria exchange fragments of plasmid DNA.