

## Bacteria

Bacteria (Sing. Bacterium) are microscopic, unicellular simplest prokaryotes, which lack chlorophyll. These cells have nucleoid instead of a true nucleus, nucleoid is not membrane bound, contains only one chromosome and its division is non-mitotic. Moreover, cells do not contain other organelles. The bacteria are smaller than fungi and measure about 0.5 to 1.0 x 2.0 to 5.0 µm in size. More than 1,600 bacterial species are known. Majority of them are saprophytes. Several species cause diseases in human beings and animals. About 200 species of bacteria cause diseases in plants. First report of plant disease by bacteria was made by **T.J. Burrill** (1878) of the University of Illinois, USA. He showed that fire blight of apple and pear is caused by a bacterium, *Erwinia amylovora*.

Bacteria have been defined by Clifton as "extremely minute, rigid essentially unicellular organisms, free of true chlorophyll and generally devoid of any photosynthetic pigments; most commonly multiplying asexually by simple transverse fission, the resulting cells being of equal or nearly equal size".

### Characteristics:

- Bacteria are unicellular organisms.
- The cells are haploid.
- Usually, they grow as a single colony.
- They are simple associations of similar cells, reproduced mainly by **binary fission**.
- They are present almost everywhere in the water, in air, in soil and inorganic bodies living or dead.
- They are prokaryotic, i.e. their DNA (genetic material) is not enclosed within a membrane and is one circular chromosome.
- Their DNA is not associated with histones (special chromosomal proteins found in eukaryotes); other proteins are associated with the DNA.
- They lack membrane-enclosed organelles.
- Mitochondria, pinocytosis, Golgi bodies, chloroplast, endoplasmic reticulum absent in the cytoplasm.
- Their cell walls almost always contain the complex polysaccharide peptidoglycan.
- They usually divide by binary fission. During this process, the DNA is copied and the cell splits into two cells.
- The various structures of a bacterial cell differ from one another not only in their physical features but also in their chemical characteristics and in their functions.

### Historical:

1676: Anton von Leeuwenhoek first observed bacteria.

1828: Christian Gottfried Ehrenberg coined the term Bacteria.

1866: **Woronin** - Described bacteria in the root nodules of leguminous plants.

1876: Louis Pasteur and Robert Koch -They proved that anthrax disease of cattle was caused by specific bacterium.

1876: Robert Koch of Germany described the theory called "Koch's postulates." He established the principles of pure culture technique.

- 1878: American Plant Pathologist -T. J. Burrill first time proved that fire blight of apple and pear was caused by a bacterium (now known as *Erwinia amylovora*).
- 1884: Christian Gram a Danish bacteriologist evolved the Gram stain technique.
- 1884: Charles Chamberland- developed porcelain bacterial filters.
- 1885: Hellriegel and Wilfarth confirmed the fixation of atmospheric nitrogen by roots of legumes with the aid of bacteria.
- 1901-1920: E.F.Smith of U.S.A gave the final proof of the fact that bacteria could be incitants of plant diseases. He also worked on the bacterial wilt of cucurbits and crown gall disease. He is also called as "**Father of Phytobacteriology**". Chilton and his coworkers demonstrated that crown gall bacterium transforms plant cell to tumour cell by introducing into them a plasmid.
- 1952: J. Lederberg coined the term plasmid
- 1952: S. A. Waksman won Nobel prize for the discovery of streptomycin.
- 1952: Zinder and J. Lederberg discovered transduction in bacteria
- 1972: P. B. New and A. Kerr success in biological control of *A. radiobacter* strain K.
- 1972: I.M.Windsor and L. M. Black observed a new kind of phloem inhabiting bacterium causing clover club leaf disease.
- 1974: I. Zanen et al. demonstrated Ti plasmid in *Agrobacterium tumefaciens*.
- 1980: D. W. Dye et al. introduced the pathovar in the taxonomy of plant pathogenic bacteria.

### Habitat of Bacteria

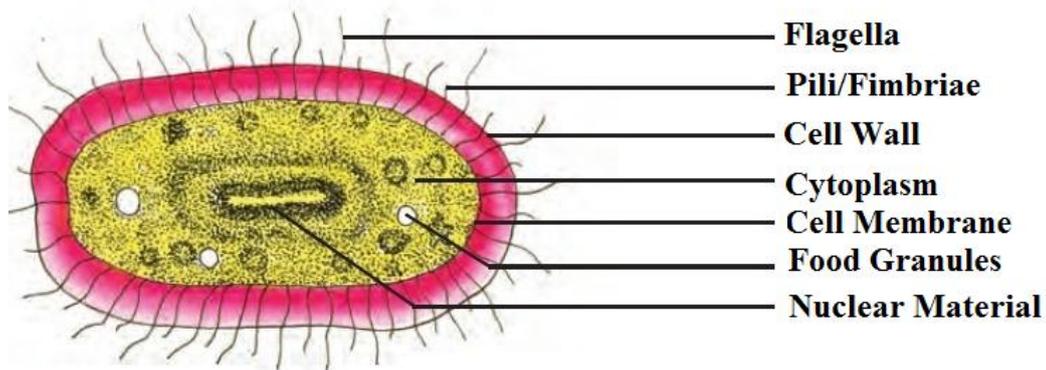
Bacteria are almost omnipresent. They are found in almost all the habitats including soil, air, water, all the living organism, animals and plants. They are very resistant and can withstand extreme cold (-190 °C), warmth (75 °C) and drought *etc.* They are normal inhabitants of human and animal intestines. They are also found in the roots of leguminous plants and keep mutualistic relationship with them. As far as their role for human life is concerned, **Louis Pasteur** has rightly stated that **without bacteria even the death will be incomplete**. Bacteria are pathogen of several diseases in animals and plants. They also used for various industrial purpose including brewing of drinks, fermentation and dairy industry. There are autotrophic bacteria also that synthesize their own food by means of photosynthesis. The pigment is not the chloroplast but it act like same. It is called **bacteriopurpurin**.

### Cell size:

Most of the bacterial cells are too small to be seen with unaided human eye and even with simple microscope. Bacteria range in size from approximately as small as the largest virus to large enough for single cells to be visible by naked eye, that is, from about 0.1 to about 600 µm over a single dimension (length or width). Normally, the dimensions of a bacterial cells is 1-5 µm (micrometre/micron: 1µm=1/1000mm) long and 0.5 µm wide, (*Cyanobacterium oscillatoria* is about 7µm in diameter -the diameter of a red blood cell) while the dimensions of a Mollicute cell like Mycoplasma/Phytoplasma (which are even smaller) is 0.1-0.4 µm or 100-400nm (nanometre: 1nm=1/1000µm or 10<sup>-9</sup>m) approximately the size of the largest viruses (poxviruses). Some rounded cells

may be as small as 0.06µm (60nm) in diameter while larger globose cells may measure up to 150-1100 nm in diameter. Filamentous forms may be from 1-2 µm to several microns in length and some spirochetes occasionally reach 500 µm in length.

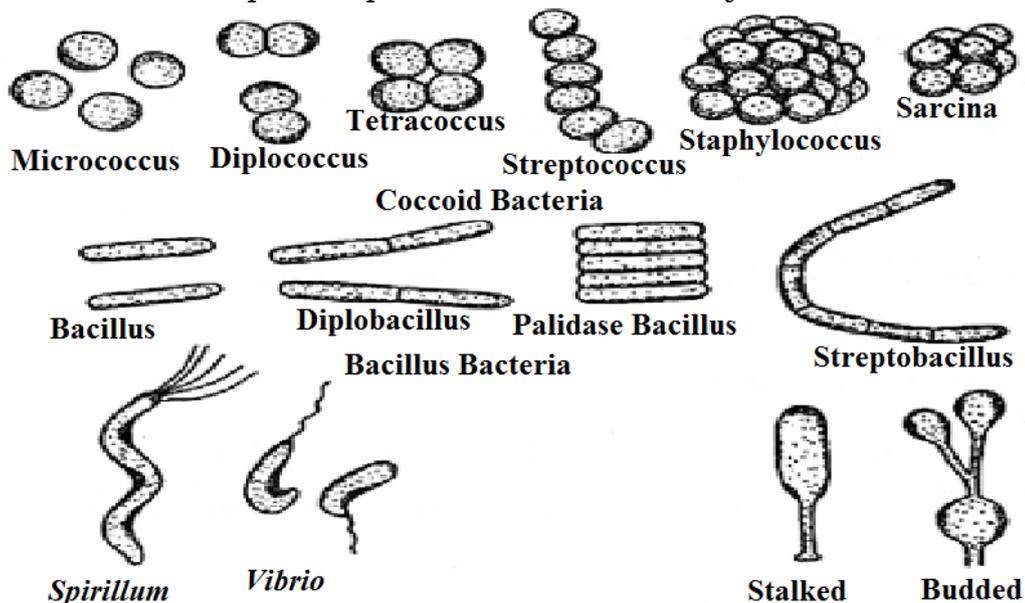
As far as size is concerned, eukaryotic cells are normally considered to be larger than the prokaryotic cells. Interestingly, the smallest eukaryotic cell is that of *Nanochlorum eukaryotum* which is only 1-2µm in diameter (about the size of *E.coli*), yet it is truly eukaryotic and has a nucleus, a chloroplast and a mitochondrion.



A Bacterial Cell

**Cell Shape:**

Cell shape is governed by presence or absence of cell wall and its chemical composition. The wall-less prokaryotes (such as Mollicutes) are **pleomorphic** (showing many shapes) due to absence of a rigid cell wall. Bacteria occur in four basic forms or shapes. The most common are spherical (**Cocci**), rod shaped (**Bacilli**), **Vibrio** and **Spiral**. The the diversity of all bacterial shapes is more difficult to explain. Though most bacterial have cells that are of a fairly constant and characteristics shape. Shape of bacterial cells may be:



VARIOUS SHAPES OF BACTERIA

1. **Spherical:** The cells which show spherical or near spherical shape are termed as **coccus (pl. cocci)**. When these are further categorized on the basis of cell arrangement, the following six categories appear:
  - a. **Monococcus:** Individual cells free of any arrangement. Only single cell represents the bacterium. Examples are *Micrococcus lutes*, *M. roseus*.
  - b. **Diplococcus:** Two cells attached to each other in pairs. Examples are *Meningococcus*, *Gonococcus*, *Diplococcus pneumoniae* etc.
  - c. **Streptococcus:** Cocci attached to one another in a chain arrangement. The chain may be varying in lengths. Example is *Streptococcus lactis*.
  - d. **Tetracoccus:** Cocci attached to one another in two planes at right angles and thus forming a group of four. Example is *Tetracoccus*.
  - e. **Staphylococcus:** Cocci in bunches as if grape bunch. In this case, cells are arranged in clusters by division of cocci in several planes. The example is *Staphylococcus aureus*.
  - f. **Sarcina (pl. sarcinae):** Cocci divided in three planes at right angles to one another and thus, forming a cubical packet (with three dimensional geometric figures) of 8 or more. Example is *Sarcina lutea*  
 However, none of the cocci have been found to be plant pathogenic. They are either human or animal pathogenic and several of them are beneficial with their industrial importance.
2. **Bacillus:** Rod-like or bullet shaped forms either singly or may be arranged differently. They are generally flagellate. **It is the most common of all the shapes. All plant pathogenic true bacteria are from this group.** Rod-shaped bacteria have variations also and these variations may be of the following types: rod, tapered rod, staff, cigar, oval and curved. Bacilli are basically longer than they are wide and they lack curvature. They typically divide only across their short axis. They are of following types:
  - a. **Monobacillus** - The bacteria occur singly. For example, *Bacillus anthrax* and *Lactobacillus* spp.
  - b. **Diplobacillus** - When bacilli are arranged in pairs, they are called diplobacilli.
  - c. **Streptobacillus** - When these cells a chain of rods e.g. *Streptobacillus* spp.
  - d. **Palisade-like** - If the cells are lined side by side like match sticks and at angles to one another. e.g. *Corynebacterium diphtheriae*.
  - e. **Coccobacillus-** A short bacillus that nearly looks like a coccus. This is a shape that exist simply to show that bacteria are so diverse that even characterizing them by shape is ambiguous. The example is *Bordetella pertussis*.
3. **Spiral bacteria-** Coiled forms of bacteria exhibiting twists with one or more turns are called spirilla. The example is *Spirillum volutes*. Flexible spirals are known as Spirochaetes (Spirochetes). The examples are *Borrelia burgdorferi* and *Treponema pallidum*.
4. **Vibrio:** Bacteria with less than one complete twists or turn are called vibrio. These resemble a comma (,) in appearance. The example of this type of shape is *Vibrio cholerae*.
5. **Stalked bacteria-** The body of bacterium possesses a stalk. *Caulobacter* is the example of this category.
6. **Budding bacteria-** The body is swollen at places looking like buds coming out of the body. The examples are *Rhodospirillum rubrum*, *Hyphomicrobium* spp.
7. **Helical bacteria-** These bacteria are helical in shape and the examples of this type of specimens include *Helicobacter pylori* (causing peptic ulcer in

human) and the notorious *Treponema pallidum* (causing syphilis). The major difference between Spiral and Helical shapes is that in spiral, the diameter is reducing (as if wire is coiled around a cone) while in helical, it remains the same (as if wire is coiled around a cylinder).

8. **Star shaped-** As the name suggests, these bacteria are shaped like a star. *Stella* spp. is one example of this shape.
9. **Mycelial forms-** These bacteria appear as if mycelium of a fungus. The examples include *Actinomyces* and *Hyphomicrobium* spp.
10. **Flat/Square shaped-** The example is Walsby's square bacterium, *Haloquadratum walsbyi*.

### **Anatomy of Bacteria:**

Any bacterial cell whether it is a coccus or a bacillus will have some structures common. These structures are cell wall, cell membrane, cytoplasm, ribosomes and the chromosome. Other intra-cellular structures such as plasmid, inclusion bodies and extra-cellular structures such as capsule, fimbriae and flagella are possessed only by some bacteria.

#### **1. Glycocalyx/Capsule/Slime:**

A gelatinous polysaccharide or polypeptide outer covering of certain bacteria is called **glycocalyx**. These are the structures that surround the outside of the cell envelope. The glycocalyx is referred to as a **capsule** if it is firmly attached to the cell wall, or as a **slime layer** if loosely attached.

The examples of capsule bacteria are *Streptococcus pneumoniae*, *Streptococcus mutans*, *Klebsiella pneumoniae*, *Bacillus anthracis*, *Neisseria meningitidis*.

#### **2. Cell wall:**

The layers of cell envelope lying between the cytoplasmic membrane and the capsule are referred to collectively as cell wall. In gram positive bacteria, the cell wall mainly consists of peptidoglycan and teichoic acid while the cell wall in gram negative bacteria includes peptidoglycan, lipoprotein, outer membrane and lipopolysaccharide layers. Cell wall does not take up any stain and hence are not seen by light microscope.

#### **Significance of cell wall:**

- Maintains cell shape, any cell that loses its cell wall, loses its shape as well.
- Protects bacteria from osmotic lysis.
- Acts as a barrier, protects cell contents from external environment
- Determines reactivity to Gram stain, cells become gram negative if they lose cell wall.
- Attachment site for flagella
- Site of action of certain antimicrobial agents (E.g. Penicillins, Cephalosporins)

#### **3. CELL MEMBRANE**

Cell membrane or cytoplasmic membrane is a typical unit membrane composed of phospholipids (40%) and proteins (60%). It measures approximately 5-10 nm in thickness. It lies below the peptidoglycan layer of the cell wall and encloses the cytoplasm.

#### **4. CYTOPLASM**

The cytoplasm or protoplasm is the portion of the cell that lies within the cytoplasmic membrane. It is gel-like in consistency and includes the prokaryotic chromosome and ribosomes. Constituents of cytoplasm include proteins (including enzymes), vitamins, ions, nucleic acids and their precursors, amino acids and their precursors, carbohydrates and their derivatives, fatty acids and their derivatives. The cytoplasm does not exhibit any internal mobility (cytoplasmic streaming). The cytoplasm also lacks organelles such as mitochondria, golgi apparatus or endoplasmic reticulum.

#### **5. Chromosome:**

The chromosome in bacteria is typically a single, closed circle DNA that is concentrated in a nucleoid region. It is not membrane bound as in eukaryotes. Some bacteria possess smaller extrachromosomal pieces of DNA called plasmids. Plasmids replicate independently of the chromosome and carry genes that are not essential for cell survival but may give some advantage to an organism. The chromosome is attached to an invagination of the cytoplasmic membrane called mesosome. Mitotic apparatus and nuclear membrane are completely lacking..

#### **6. Ribosomes:**

Bacterial cells can contain thousands of ribosomes, which are the sites of protein synthesis. The distinct granular appearance of procaryotic cytoplasm is due to the presence and distribution of ribosomes. Often they aggregate to form structures known as polysomes. Bacterial ribosomes are termed 70 S (Svedberg units) and eukaryotic ribosomes are termed 80S. The difference between bacterial and eukaryotic ribosomes is often exploited during antibiotic therapy.

#### **7. Inclusion bodies:**

Intracytoplasmic inclusions can be vacuoles, crystals or storage bodies. Bacteria often store reserve material in the form of insoluble cytoplasmic granules. Inclusions accumulate when a cell is grown in the presence of excess nutrients and they are often observed under laboratory conditions. Various examples of these bodies are:

- Starch/Glycogen granules - blue-greens and enteric bacteria
- Poly- $\beta$ -hydroxybutyrate granules - *Azotobacter* and *Rhizobium*
- Nitrogen-reserve granules - blue-greens
- Sulphur inclusions – *Thiotrix*
- Lipid inclusions
- Volutin granules – *Corynebacterium diphtheria*

#### **8. FLAGELLA:**

Some bacteria are motile and some are not. Almost all motile bacteria possess flagella as the organ of locomotion. Such bacteria tend to move towards or away from the source of stimulus. These stimuli can be chemicals (chemotaxis), light (phototaxis), air (aerotaxis) or magnetism (magnetotaxis).

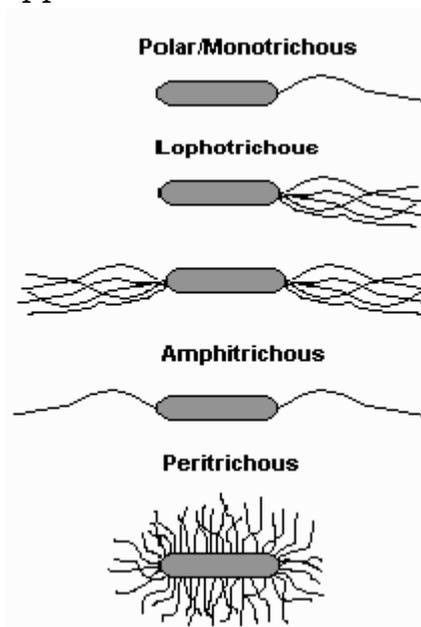
##### **Structure:**

Procaryotic flagella are much thinner than eukaryotic flagella and they lack the typical 9 + 2 arrangement of microtubules. Over 40 genes are involved in its assembly and function. They are approximately 3-20 $\mu$ m long and end in a square

tip. Since flagella are very thin (20-30 nm in diameter), they are below the resolution limits of a normal light microscope and cannot be seen. The bacterial flagellum is a non contractile, composed of single kind of protein subunit called flagellin. It is anchored to the bacterial cytoplasmic membrane and cell wall by means of disk like structures. The numbers of flagella, as well as their location on the cell surface are characteristic of a species.

**Flagella arrangements are:**

1. **Monotrichous** - a single flagellum at one pole (also called polar flagellum) E.g. *Xanthomonas* spp., *Vibrio cholerae*
2. **Amphitrichous** - single flagellum at both poles. Eg. *Pseudomonas* spp., Spirilla
3. **Lophotrichous** - two or more flagella at one or both poles of the cell E.g. *Spirillum undula*
4. **Peritrichous** - completely surrounded by flagella E.g. *Erwinia* spp., *E.coli*
5. **Atrichous** - There is no flagellum found on the cell surface. The cell is, in fact, non- flagellate. The examples are all the cocci and few bacilli such as *Lactobacillus* spp.



Other mechanisms of bacterial locomotion include gliding and motion by axial filament contraction. Gliding is movement of bacteria along solid surfaces by an unknown mechanism. Spirochetes have internally-located axial filaments or endoflagella. Axial filaments wrap around the spirochete towards the middle from both ends. They are located above the peptidoglycan cell wall but below the outer membrane.

## 9. FIMBRIAE AND PILI:

Fimbriae are short, hair-like structures made up of protein pilin and are present in many gram negative bacteria. Even though pili arise from plasma membrane they are not considered part of the plasma membrane. They are anchored in the membrane and protrude through the cell wall to the outside of the cell. Fimbriae are shorter and straighter than flagella and are more numerous. They are 0.5µm long and 10 nm thick. Since they are made up of protein, they are antigenic. Bacteria from different genera may possess common fimbrial

antigens. Fimbriae are usually seen in young cultures and lost on subcultures on solid media. While some authors use the two terms (fimbriae and pili) interchangeably, some restrict the term pili to denote sex pili. Sex pili acts to join bacterial cells for transfer of DNA from one cell to another by a process called conjugation.

#### 10. SPORE:

In poor growth conditions some bacteria such as *Bacillus* and *Clostridium* produce resistant survival forms termed **endospores**. This process is known as **sporulation**. **Bacterial spores are endospores in contrast to fungal spores, which are usually exospores. Unlike the spores of fungi, bacterial spores do not serve reproductive function.** They are resistant to extreme environmental conditions such as high temperatures, dryness, toxic chemicals (disinfectants, antibiotics), and UV radiation. Once the endospore is formed, the vegetative portion of the bacterium is degraded and the dormant endospore is released. The endospore is able to survive for long periods of time until environmental conditions again become favorable for growth. The endospore then germinates, producing a single vegetative bacterium. Spores can be killed by sterilization methods such as autoclave and hot air oven. Some chemical disinfectants such as formaldehyde and ethylene oxide can also kill spores.

A **bacterial species** is a group of bacterial strains that share certain phenotypic and genotypic characteristics. One of these strains serves as the type strain, with the other strains of the species differing to a lesser or greater extent from the type strain. Bacterial strains may differ from each other in morphological, cultural, physiological, biochemical or pathological characteristics. When a strain or group of strains infects a host plant not infected by the other strains of the species, that strain or group of strains comprises a **pathovar** (pv.) of the species. Accordingly, each species may have several pathovars, each (single or group of strains) able to infect a particular host plant.

About 1600 species of bacteria are known. Most of these are saprophytes. Some species cause diseases in humans and about 100 species cause diseases in plants. Most plant pathogenic bacteria are facultative saprophytes and can be easily grown on nutrient media in the laboratory.

#### **Characteristics of Plant Pathogenic Bacteria:**

Most Plant pathogenic bacteria are rod-shaped, except those of the genus *Streptomyces* which are filamentous. **Most Phytopathogenic genera are Gram-negative**, except *Clavibacter* (formerly *Corynebacterium*) and *Streptomyces*, which are Gram-positive. Most move actively by flagella.

In young cultures bacterial rods range from 0.6×3.5 μm in size. The cell walls are enveloped by a definite capsule or diffuse slime layer. In most plant pathogenic genera cells have polar (at one end of the cell) flagellum or they may be peritrichous (flagella over entire cell surface). They have thin, relatively tough, rigid cell walls and an inner cell membrane. The material inside the cell wall, i.e. protoplast consists of cytoplasmic membrane, cytoplasm and the nuclear material (a large circular chromosome composed of DNA, diffused in the cytoplasm). Rod-shaped phytopathogenic bacteria reproduce by binary fission or fission at rapid rate, one cell producing one million progeny in less than a day.

The main characteristics of the most common plant pathogenic bacteria, helpful in their identification are as follows:

**(A) Gram negative Genera:**

1. **Agrobacterium:** Rod-shaped,  $0.8 \times 1.5-3.0 \mu\text{m}$  in size, motile by one to four, sometimes up to six peritrichous flagella, Gram-negative and aerobic. Usually gall-forming pathogens causing cortical hypertrophy except *A. radiobacter*. The cells are without endospores.
2. **Erwinia:** Predominantly single and straight rods measuring  $0.5-1.0 \times 1.0-3.0 \mu\text{m}$  in size. The bacteria are motile by several to many peritrichous flagella except *E. stewarti* (now named *Pantoea stewarti* subsp. *stewarti*) and *E. dissolvens* (now named as *Enterobacter dissolvens*). This is the only phytopathogenic bacterial genus with members being facultative anaerobes. Some species do not produce pectic enzymes and cause necrotic or wilt diseases (amylovora group) such as *E. amylovora*, *E. chrysanthemi* and *E. tracheiphila*. Others are strongly pectolytic and cause soft rots (carotovora group). A third group also exists which consists of epiphytes that cause neither soft rots nor necrosis (including *E. herbicola*=*Enterobacter agglomerans*) and saprophytes like *E. persicinus*. These are Gram negative bacteria.
3. **Pseudomonas:** Cells are single and straight to curved rods with dimensions of  $0.5-1.0 \times 1.5-4.0 \mu\text{m}$ . They are motile by one or many polar flagella. Most are phytopathogenic while a few infect animals and human. They are non-spore forming and strictly aerobic in nature. These Gram negative bacteria are have been accommodated into the following groups on the basis of production of fluorescent pigment on iron-deficient media and poly- $\beta$ -hydroxybutyrate inclusions.
  - a. **The Fluorescent group:** The group contains species that mostly produce fluorescent pigment with most phytopathogenic members belonging to *Pseudomonas syringae* and its pathovars.
  - b. **The Solanaceous group:** Many non-fluorescent pathogenic species including *Pseudomonas solanacearum* (renamed as *Burkholderia solanacearum* and then as *Ralstonia solanacearum*).
  - c. **The Acidovorans group:** The species under this group have been considered as the members of a single species, *Pseudomonas avenae*, with some being its synonyms while others being its pathovars and later transferred to the genus *Acidovorax*. Alongwith *P. avenae*, the group includes *P. rubrilineans* also.
4. **Ralstonia:** These are rod shaped, motile, gram negative bacteria which are mainly soil inhabitants. *Ralstonia* is a complex genus with 2 pathogenic species. *R. solanacearum* is a very complex species. The species is known to cause wilts of solanaceous crops. Another species is *R. syzygi*.
5. **Xanthomonas:** The genus causing numerous leaf spots, fruit spots, blights of annual and perennial plants, vascular wilts and citrus canker. They are slow growing in nature and all the species are plant pathogenic and are found only in association with plants or plant material. They are also rod-shaped and motile.

- 6. Xylella:** Single straight rods, non-motile, gram negative, aerobic, nutritionally fastidious. As stated earlier, the habitat of *Xylella* is xylem of plant tissue. They are known to cause wilt diseases.

**(B) Gram positive Genera:**

- 7. Clavibacter:** Formerly known as *Corynebacterium*, this phytopathogenic genera consists of straight to slightly curved (pleomorphic) rods measuring  $0.5-0.9 \times 1.5-4.0 \mu\text{m}$  in size. They are generally nonmotile but some may be motile with one or two polar flagella. The *Corynebacterium tritici* has been reclassified as *Rathayibacter tritici*.

- 8. Curtobacterium:** The cells are small short rods but in old cultures coccoid cells may be seen. The cells multiply by bending type of cell division and are generally motile by lateral flagella.

**Plant Pathogenic species:** *Curtobacterium floccumfaciens* pv. *floccumfaciens*, *Curtobacterium floccumfaciens* pv. *betae*, *Curtobacterium floccumfaciens* pv. *oortii* and *Curtobacterium floccumfaciens* pv. *poinsettiae*.

- 9. Rhodococcus:** The species *fascians* of genus *Corynebacterium* has been raised to a separate generic level with the name *Rhodococcus fascians*. The bacterium causes malformation of aerial plant parts (a symptom called fasciation, on which the species was named). Cells in young cultures measure  $0.5-0.9 \times 1.5-4.0 \mu\text{m}$ . Nonmotile, slightly curved rods with the cells arranged singly, at an angle or in a palisade manner.

- 10. Arthrobacter:** This genus is suspected to be *Curtobacterium*.

- 11. Streptomyces:** The genus comprises of widely distributed soil inhabitants. The morphology is filamentous with slender branched hyphae without cross walls. At maturity the aerial mycelium forms chains of three to many spores. The bacterium is Gram positive and causes scab diseases in many plant species.