

CHAPTER 05: STUDY of the MAJOR BACTERIAL GROUPS

5.3. GRAM-NEGATIVE HETEROTROPHIC BACTERIA

1. Introduction

Most of the bacterial species are **heterotrophic** in nature i.e. utilize nutrition from other living being. Though they lack the photosynthetic pigment they are unable to utilize solar energy. These bacteria with the help of enzymes convert the complex organic compounds in soluble form and absorb them. These bacteria are classified into three types:

a. Saprophytic Bacteria: they survive on dead and deteriorating organic compound. These bacteria absorb nutrition from them. Firstly, they convert the complex organic compound into soluble compound with the help of enzymes and then absorb them according to their requirement or absorb them conditionally.

b. Symbiotic bacteria: those bacteria which grow and develop in close beneficial partnership or association with other living organism are called symbiotic bacteria and the phenomenon is termed as *symbiosis*. For example, these bacteria occur in the root nodules of leguminous plants where they fix free atmospheric nitrogen in the soil which is utilized by plants and plants in turn provide them carbohydrate and shelter for proper development, e.g. *Rhizobia* spp.

c. Parasitic bacteria: those bacteria which feed themselves on living tissues (host) are called parasitic bacteria. They are transmitted to the host by means of air, water and food. These bacteria may be obligate parasite or facultative parasite or may pathogenic or non-pathogenic.

2. Phylum *Spirochaetes*

The phylum *Spirochaetes* (Greek *Spira* = a coil, and *chaete* = hair) contains **Gram-negative**, chemoheterotrophic bacteria distinguished by their structure and mechanism of motility. They are slender, long bacteria (0.1 to 3.0 mm by 5 to 250 nm) with a flexible, helical shape (Fig. 01).

Spirochetes can be anaerobic, facultatively anaerobic, or aerobic. Carbohydrates, amino acids, long-chain fatty acids, and long-chain fatty alcohols may serve as carbon and energy sources.

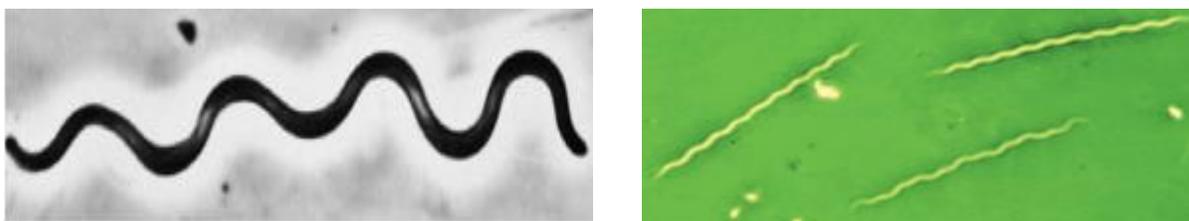


Figure 01: Spirochetes.

[*Cristispira* sp. from a clam; phase contrast (x 2,200) (in left), *Treponema pallidum* (x 1,000) (in right)]

The distinctive features of spirochete morphology are evident in electron micrographs (Fig. 02). The central protoplasmic cylinder contains cytoplasm and the nucleoid, and is bounded by a plasma membrane and a Gram-negative cell wall. Two to more than a hundred flagella, called **axial fibrils**, **periplasmic flagella**, or **endoflagella**, extend from both ends of the cylinder and often overlap one another in the center third of the cell. The whole complex of periplasmic flagella, the **axial filament**, lies inside a flexible outer sheath. The outer sheath contains lipid, protein, and carbohydrate and varies in structure between different genera. Its precise function is unknown, but the sheath is essential because spirochetes die if it is damaged or removed. The outer sheath of *Treponema pallidum* has few proteins exposed on its surface. This allows the syphilis spirochete to avoid attack by host antibodies.

Spirochetes differ greatly from other bacteria with respect to motility and can move through very viscous solutions though they lack external rotating flagella. When in contact with a solid surface, they exhibit creeping or crawling movements. Their unique pattern of motility is due to an unusual morphological structure called the **axial filament**. The periplasmic flagella rotate like the external flagella of other bacteria. This causes the corkscrew-shaped outer sheath to rotate and move the cell through the surrounding liquid (Fig. 03). Flagellar rotation may also flex or bend the cell and account for the crawling movement seen on solid surfaces.

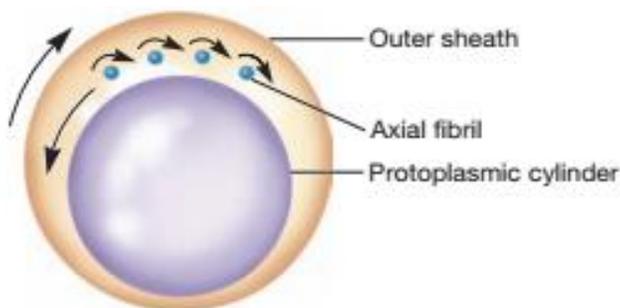


Figure 03: Spirochete Motility (A hypothetical mechanism for spirochete motility).

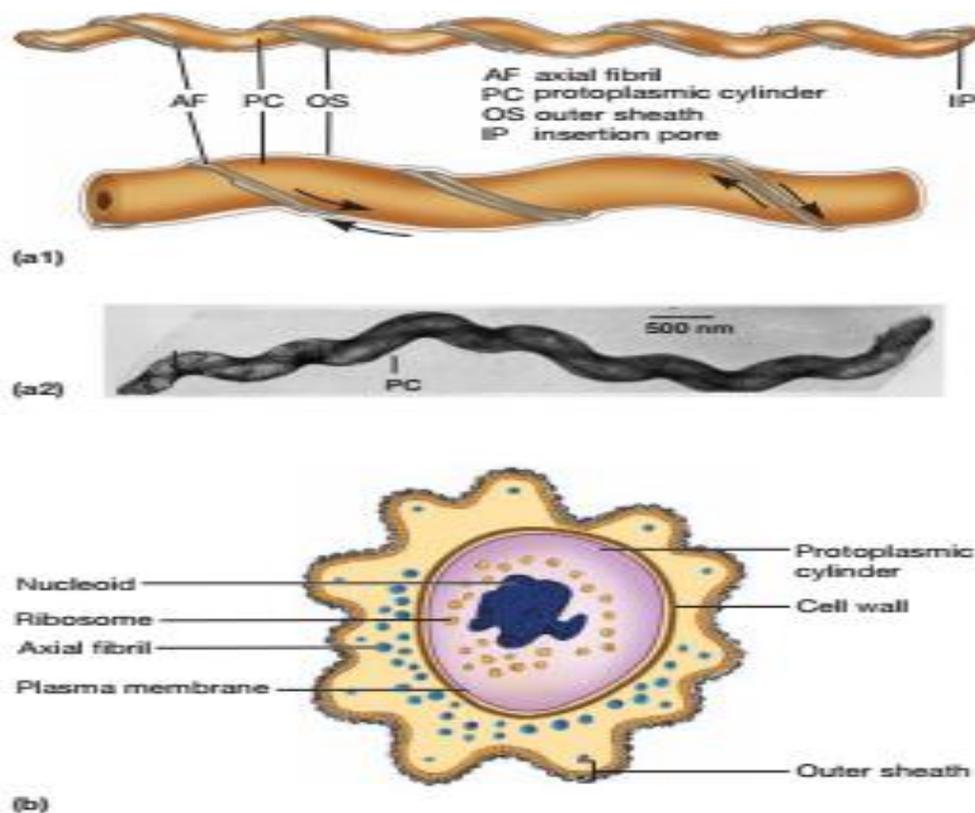


Figure 02: Spirochete morphology.

[(a1) A surface view of spirochete structure as interpreted from electron micrographs, (a2) A longitudinal view of *Treponema zuelzeriae* with axial fibrils extending most of the cell length, (b) A cross section of a typical spirochete showing morphological details].

The group is exceptionally diverse ecologically and grows in habitats ranging from mud to the human mouth. Members of the genus *Spirochaeta* are free-living and often grow in anoxic and sulfide-rich freshwater and marine environments. Some species of the genus *Leptospira* grow in oxic water and moist soil. Some spirochetes form symbiotic associations with other organisms and are found in a variety of locations: the hindguts of termites and wood-eating roaches, the digestive tracts of mollusks (*Cristispira*) and mammals, and the oral cavities of animals (*Treponema denticola*, *T. oralis*). Spirochetes from termite hindguts and freshwater sediments have nitrogenase and can fix nitrogen. Spirochetes coat the surfaces of many protozoa from termite and wood-eating roach hindguts. For example, the flagellate *Myxotricha paradoxa* is covered with slender spirochetes (0.15 by 10 mm in length) that are firmly attached and help move the protozoan.

Some members of the genera *Treponema*, *Borrelia*, and *Leptospira* are important pathogens. *Treponema pallidum* causes syphilis, and *Borrelia burgdorferi* is responsible for Lyme disease. The study of *T. pallidum* and its role in syphilis has been hindered by the inability to culture the

spirochete outside its human host. Its genome sequence shows that this spirochete is metabolically crippled and quite dependent on its host. The *B. burgdorferi* genome consists of a linear chromosome and at least 17 linear and circular plasmids (plasmid proteins seem to be involved in bacterial virulence).

As a result of the current analyses of 16S rRNA gene sequences, *Bergey's Manual* divides the phylum *Spirochaetes* into one class (*Spirochaetia*) and one order (*Spirochaetales*), which is presently comprised of four families, namely *Spirochaetaceae*, *Brachyspiraceae*, *Brevinemataceae*, and *Leptospiraceae*.

Example of classification:

Domain: Bacteria	Domain: Bacteria
Phylum: Spirochaetes	Phylum: Spirochaetes
Class: Spirochaetia	Class: Spirochaetia
Order: Spirochaetales	Order: Spirochaetales
Family: Spirochaetaceae	Family: Leptospiraceae
Genus: <i>Spirochaeta</i> , <i>Borrelia</i> , <i>Cristispira</i> and <i>Treponema</i>	Genus: <i>Leptospira</i> , <i>Leptonema</i> and <i>Turneriella</i>

3. Phylum *Bacteroidetes*

The phylum *Bacteroidetes* is a phenotypically diverse group of Gram-stain-negative rods that do not form endospores. *Bergey's manual* classified the members of this phylum on the basis of phylogenetic analysis of 16S rRNA gene sequences. The phylum contains four classes, *Bacteroidia*, *Cytophagia*, *Flavobacteriia*, and *Sphingobacteria*. No unifying phenotypic feature characterizes this diverse group, but their phylogenetic closeness causes them to be placed together.

The class *Bacteroidia* contains anaerobic, **Gram-negative**, non-sporing, motile or nonmotile rods of various shapes. These bacteria are chemoheterotrophic and usually produce a mixture of organic acids as fermentation end products. They do not reduce sulfate or other sulfur compounds. The genera are identified using properties such as general shape, motility and flagellation pattern, and fermentation end products. These bacteria grow in habitats such as the oral cavity and intestinal tract of vertebrates and the rumen of ruminants.

Although difficulty culturing these anaerobes has hindered our understanding of them; they are clearly widespread and important. Often, they benefit their host. *Bacteroides ruminicola* is a

major component of the rumen flora; it ferments starch, pectin, and other carbohydrates. About 30% of the bacteria isolated from human feces are members of the genus *Bacteroides*, and these organisms may provide extra nutrition by degrading cellulose, pectin, and other complex carbohydrates. Members of the genus *Bacteroides* are associated with diseases of major organ systems, ranging from the central nervous system to the skeletal system. *Bacteroides fragilis* is a particularly common anaerobic pathogen found in abdominal, pelvic, pulmonary, and blood infections.

The class *Flavobacteriia* includes only the order *Flavobacteriales*. This order contains the families *Flavobacteriaceae* (type genus: *Flavobacterium*), *Blattabacteriaceae*, and *Cryomorphaceae*. It consists of Gram-stain-negative, non-spore forming rods or filaments devoid of gas vesicles and intracellular granules of poly- β -hydroxybutyrate, which usually multiply by binary fission; ring-shaped cells are not formed.

The class *Sphingobacteriia* comprises morphologically diverse non-spore forming bacteria that stain Gram-negative. Growth is aerobic or facultatively anaerobic, and a yellow pigmentation is often formed. The class contains one order, *Sphingobacteriales*. The order contains two families, *Sphingobacteriaceae* (type genus: *Sphingobacterium*) and *Chitinophagaceae* (type genus: *Chitinophaga*). All genera studied for this feature (mainly *Sphingobacterium*) contain high concentrations of sphingophospholipids as cellular lipid components.

The members of the class *Cytophagia* are short or long rods and sometimes filaments. Some genera form rings, coils, vibroids or S-shaped cells. Spores or other resting cells are not found except for the genus *Sporocytophaga*. Motile by gliding or flagella, or nonmotile. Flagella are found in only the genus *Balneola*. **Stain Gram-negative**. Growth is usually strictly aerobic, but microaerobic to anaerobic growth occurs in a few members. **Chemo-organotrophic**. Colonies are usually pigmented, and cell masses are yellow, orange, pink, or red owing to carotenoids, flexirubin-type pigments, or both. Widely distributed in nature. Some genera are marine organisms that require seawater salts for growth. Most species are mesophilic, but psychrophilic and thermophilic members exist. The class includes the type order *Cytophagales* and some other deep phylogenetic groups. The order is circumscribed for this volume (Volume 04 of Bergey's manual 2ⁿ edition) to encompass the three families *Cytophagaceae* (type genus: *Cytophaga*), *Cyclobacteriaceae*, and *Flammeovirgaceae*, based on 16S rRNA sequences. The families *Cyclobacteriaceae* and *Flammeovirgaceae* were not described in previous editions of the manual.

Because the three families belonging to the order *Cytophagales* share many phenotypic characteristics, they are difficult to differentiate based solely on phenotype.

The genera *Cytophaga*, *Sporocytophaga*, and *Flexibacter* (family *Cytophagaceae*) differ from each other in morphology, life cycle, and physiology. Bacteria of the genus *Cytophaga* are slender rods, often with pointed ends. *Sporocytophaga* is similar to *Cytophaga* but forms spherical resting cells called microcysts. *Flexibacter* produces long, flexible threadlike cells when young and is unable to use complex polysaccharides. Often colonies of these bacteria are yellow to orange because of carotenoid or flexirubin pigments.

Members of the genera *Cytophaga* and *Sporocytophaga* are aerobes that actively degrade complex polysaccharides. Soil cytophagas digest cellulose; both soil and marine forms attack chitin, pectin, and keratin. Some marine species even degrade agar, a component of seaweed. Cytophagas play a major role in the mineralization of organic matter and can cause great damage to exposed fishing gear and wooden structures. Although most cytophagas are free-living, some can be isolated from vertebrate hosts and are pathogenic. *Cytophaga columnaris* and others cause diseases such as columnaris disease, cold water disease, and fin rot in freshwater and marine fish.

The gliding motility so characteristic of these organisms is quite different from flagellar motility. Gliding bacteria lack flagella and are stationary while suspended in liquid medium. The movement can be very rapid and when the bacteria is in contact with a surface, they glide along leaving a slime trail. Young organisms are the most motile, and motility often is lost with age. Low nutrient levels usually stimulate gliding. The gliding mechanism is not well understood. Gliding bacteria, like flagellated bacteria, can position themselves at optimal conditions of light intensity, oxygen, hydrogen sulfide, temperature, and other factors that influence growth and survival.

Example of classification:

Domain: Bacteria	Domain: Bacteria
Phylum: Bacteroidetes	Phylum: Bacteroidetes
Class: Bacterioidia	Class: Cytophagia
Order: Bacteroidales	Order: Cytophagales
Family: Bacteroidaceae	Family: Cytophagaceae
Genus: Bacteroides	Genus: <i>Cytophaga</i>
Species: <i>Bacteroides fragilis</i>	Species: <i>Cytophaga columnaris</i>
