CHAPTER 05: STUDY of the MAJOR BACTERIAL GROUPS

5.4. GRAM-POSITIVE HETEROTROPHIC BACTERIA

1. Introduction

Gram-positive bacteria were historically grouped on the basis of their general shape (e.g., rods, cocci, or irregular) and their ability to form endospores. However, analysis of the phylogenetic relationships within the Gram-positive bacteria by comparison of 16S rRNA sequences shows that they are divided into a low G + C group and high G + C. The second edition of *Bergey's Manual of Systematic Bacteriology* divided the Gram-positive bacteria into two large phyla, the *Firmicutes* which placed in volume 3 (low G + C content, under 50 per cent) and the *Actinobacteria* (high G + C content, over 50 per cent). Gram-positive bacteria mostly have a chemoheterotrophic mode of nutrition and include among their number several important human pathogens, as well as industrially significant forms. The phylum *Firmicutes* are divided into three classes: *Clostridia*, *Mollicutes*, and *Bacilli* with 10 orders and 33 families.

To illustrate the variations in G + C ratio, the genera *Clostridium* and *Streptococcus* have a low G + C content with 21-54% and 33-34% respectively, whereas, the genus *Streptomyces* have a high G + C content of 69-73%.

2. Class *Clostridia*

The class *Clostridia* has a very wide variety of Gram-positive bacteria distributed into three orders and 11 families. The largest genus is *Clostridium.* It includes obligately anaerobic, fermentative, Gram-positive bacteria that form endospores. The genus contains well over 100 species in several distinct phylogenetic clusters.

Members of the genus *Clostridium* have great practical impact. Because they are anaerobic and form heat-resistant endospores, they are responsible for many cases of food spoilage, even in canned foods. *C. botulinum* is the causative agent of botulism. Clostridia often can ferment amino acids to produce ATP by oxidizing one amino acid and using another as an electron acceptor (Stickland reaction) and generate ammonia, hydrogen sulfide, fatty acids, and amines during the anaerobic decomposition of proteins.

Several clostridia produce toxins and are major disease agents. *C. tetani* (Fig. 01) is the causative agent of tetanus, and *C. perfringens,* of gas gangrene and food poisoning. *C. perfringens* genome sequence analysis reveals that the microbe possesses the genes for fermentation with gas production but lacks genes encoding enzymes for the TCA cycle or a respiratory chain. Nonetheless, *C. perfringens* has an extraordinary doubling time of only 8 to 10 minutes when in the human host. Clostridia also are industrially valuable; for example, *C. acetobutylicum* is used to manufacture butanol in some countries.

Desulfotomaculum is another anaerobic, endospore-forming genus. Unlike *Clostridium,* it reduces sulfate and sulfite to H_2S during anaerobic respiration. Although it stains Gram negative, electron microscopic studies have shown that *Desulfotomaculum* has a Gram-positive type cell wall. This concurs with phylogenetic studies that place it with the low G+C Gram positives.

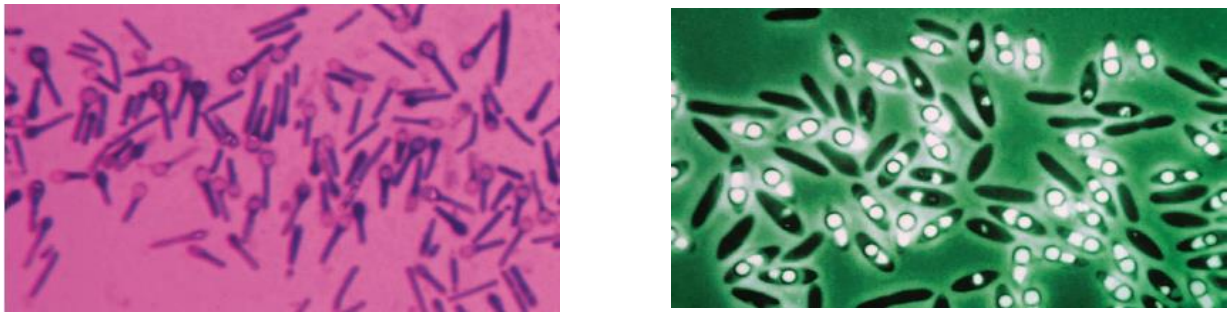

Figure 01: *Clostridium tetani* (in left with spores that are round and terminal) and *Desulfotomaculum acetoxidans* (in right with spores; phase contrast (32,000).

3. Class *Bacilli*

The second edition of *Bergey's Manual* gathers a large variety of Gram-positive bacteria into one class, *Bacilli,* and two orders, *Bacillales* and *Lactobacillales.* These orders contain 17 families and over 70 Gram-positive genera representing cocci, endospore-forming rods and cocci, and nonsporing rods.

3.1. Order *Bacillales*

3.1.1. Family *Bacillaceae*

The genus *Bacillus* is the largest in the order (Fig. 02). The genus contains Gram-positive, endospore forming, chemoheterotrophic rods that are usually motile with peritrichous flagella. It is aerobic, or sometimes facultative, and catalase positive.

Bacillus subtilis, type species, is a useful model organism for the study of gene regulation, cell division, quorum sensing, and cellular differentiation. Its 4.2-Mb genome was one of the first genomes to be completely sequenced, which reveals a number of interesting elements. For instance, several families of genes have been expanded by gene duplication; the largest such family encodes ABC transporters, which are the most frequent type of protein in *B. subtilis*. The genome contains genes for the catabolism of many diverse carbon sources and antibiotic synthesis.

Many species of *Bacillus* are of considerable importance. Some produce the antibiotics bacitracin, gramicidin, and polymyxin. *B. cereus* (Fig. 02) causes some forms of food poisoning and can infect humans. *B. anthracis* is the causative agent of the disease anthrax, which can affect both farm animals and humans.

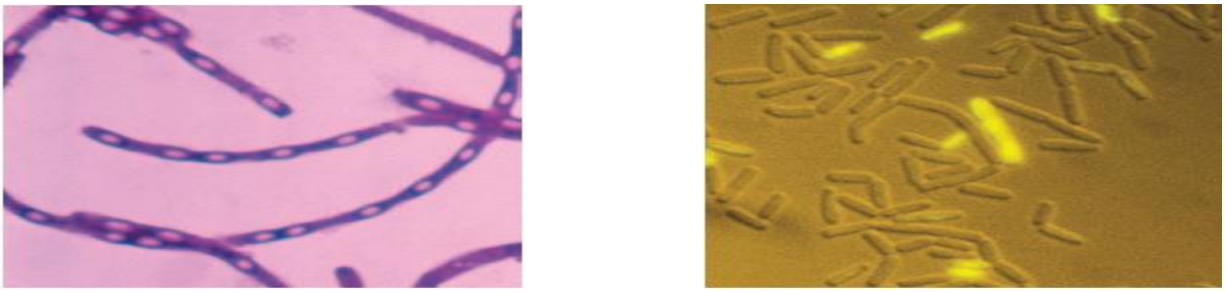

Figure 02: *Bacillus*.
[in left *B. anthracis*: spores elliptical and central (31,600). (in right *B. cereus* stained with SYTOX Green nucleic acid stain and viewed by epifluorescence and differential interference contrast microscopy. The cells that glow green are dead].

Several species are used as insecticides. *B. thuringiensis* and *B. sphaericus* form a solid protein crystal, the **parasporal body**, next to their endospores during spore formation. The *B. thuringiensis* parasporal body contains protein toxins that kill over 100 species of moths by dissolving in the alkaline gut of caterpillars and destroying the epithelium. *B. thuringiensis* toxin genes have been engineered to make a variety of pest-resistant, genetically modified plants. The *B. sphaericus* parasporal body contains proteins toxic for mosquito larvae and may be useful in controlling the mosquitos that carry the malaria parasite *Plasmodium.*

3.1.2. Family *Staphylococcaceae*

This family contains four genera, the most important of which is the genus *Staphylococcus*. Members of this genus are facultatively anaerobic, nonmotile, Gram-positive cocci that usually form irregular clusters (Fig. 03). They are catalase positive, oxidase negative, ferment glucose, and

have teichoic acid in their cell walls. Staphylococci are normally associated with the skin, skin glands, and mucous membranes of warm-blooded animals.

Staphylococci are responsible for many human diseases. *S. epidermidis* is a common skin resident that is sometimes responsible for endocarditis and infections of patients with lowered resistance (e.g., wound infections, surgical infections, urinary tract infections, body piercing). The growth and hemolysis patterns on blood agar are useful in identifying this staphylococcus (Fig. 03).

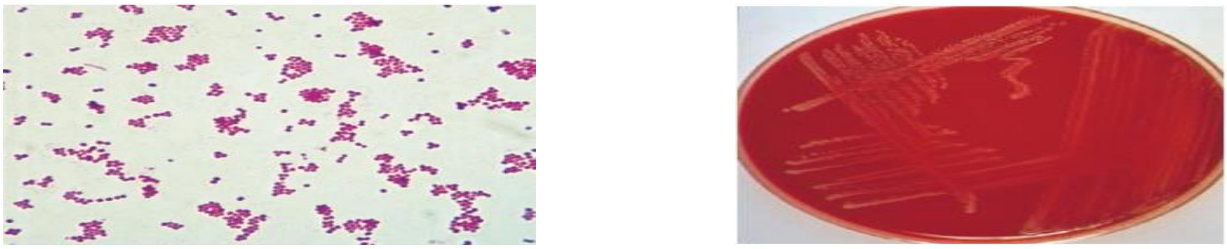

Figure 03: *Staphylococcus*.
[in left *Staphylococcus aureus*, Gram-stained smear (X1,500), in right *Staphylococcus epidermidis* on blood agar with no hemolysis].

S. aureus is the most important human staphylococcal pathogen and causes boils, abscesses, wound infections, pneumonia, toxic shock syndrome, and other diseases. Strains of **meticillin-resistant *Staphylococcus aureus*** (**MRSA**: formerly methicillin) and vancomycin-resistant *S. aureus* are among the most threatening antibiotic-resistant pathogens known. Vancomycin is considered the "drug of last resort" and infections caused by vancomycin-resistant *S. aureus* generally cannot be treated by antibiotic therapy.

One of the virulence factors produced by *S. aureus* is the enzyme **coagulase,** which causes blood plasma to clot. *S. aureus* usually grows on the nasal membranes and skin; it also is found in the gastrointestinal and urinary tracts of warm-blooded animals. It's also a major cause of food poisoning.

3.1.3. Family *Listeriaceae*

Listeria contains short rods that are aerobic or facultative, catalase positive, and motile by peritrichous flagella. It is widely distributed in nature, particularly in decaying matter. *Listeria monocytogenes* is a pathogen of humans and other animals and causes listeriosis, an important food infection.

3.2. Order *Lactobacillales*

Many members of the order *Lactobacillales* produce lactic acid as their major or sole fermentation product and are sometimes collectively called **Lactic Acid Bacteria (LAB)**. *Streptococcus, Enterococcus, Lactococcus, Lactobacillus,* and *Leuconostoc* are all members of this group. Lactic acid bacteria are non-sporing and usually nonmotile. They normally depend on sugar fermentation for energy. They lack cytochromes and obtain energy by substrate-level phosphorylation rather than by electron transport and oxidative phosphorylation. Nutritionally, they are fastidious and many vitamins, amino acids, purines, and pyrimidines must be supplied because of their limited biosynthetic capabilities. Lactic acid bacteria usually are categorized as facultative anaerobes, but some classify them as aerotolerant anaerobes.

3.2.1. Family *Lactobacillaceae*

The largest genus in this order is *Lactobacillus* with around 100 species. *Lactobacillus* contains non-sporing rods and sometimes coccobacilli that lack catalase and cytochromes, are usually facultative anaerobic or microaerophilic, produce lactic acid as their main or sole fermentation product, and have complex nutritional requirements (Fig. 04). Lactobacilli carry out either a homolactic fermentation using the Embden-Meyerhof pathway or a heterolactic fermentation with the pentose phosphate pathway. They grow optimally under slightly acidic conditions (pH = 4.5-6.4). The genus is found on plant surfaces and in dairy products, meat, water, sewage, beer, fruits, and many other materials. Lactobacilli also are part of the normal flora of the human body in the mouth, intestinal tract, and vagina. They usually are not pathogenic.

Lactobacillus is indispensable to the food and dairy industry. Lactobacilli are used in the production of fermented vegetable foods, beverages (beer, wine, juices), sour dough bread, Swiss and other hard cheeses, yogurt, and sausage. Yogurt is probably the most popular fermented milk product in the United States using *Streptococcus thermophilus* and *Lactobacillus bulgaricus.* Acting together, the two species ferment almost all the lactose to lactic acid and flavor the yogurt with diacetyl (*S. thermophilus*) and acetaldehyde (*L. bulgaricus*).

At least one species, *L. plantarum,* is sold commercially as a probiotic agent that may provide some health benefits for the consumer. On the other hand, some lactobacilli also create problems. They sometimes are responsible for spoilage of beer, milk, and meat because their metabolic end products contribute undesirable flavors and odors.

3.2.2. Family *Leuconostocaceae*

Leuconostoc contains facultative Gram-positive cocci, which may be elongated or elliptical and arranged in pairs or chains (Fig. 04). Leuconostocs lack catalase and cytochromes and carry out heterolactic fermentation by converting glucose to D-lactate and ethanol or acetic acid by means of the phosphoketolase pathway. They can be isolated from plants, silage, and milk. The genus is used in wine production, in the fermentation of vegetables such as cabbage (sauerkraut) and cucumbers (pickles), and in the manufacture of buttermilk, butter, and cheese. *L. mesenteroides* synthesizes dextrans from sucrose and is important in industrial dextran production. *Leuconostoc* species are involved in food spoilage and tolerate high sugar concentrations so well that they grow in syrup and are a major problem in sugar refineries.

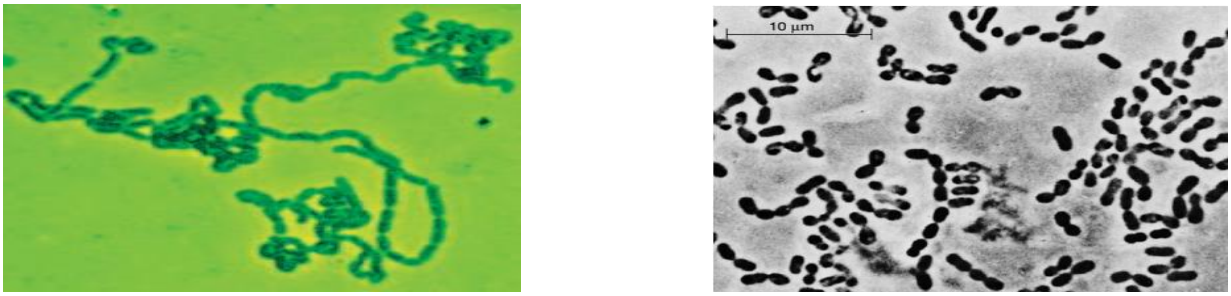

Figure 04: *Lactobacillus* and *Leuconostoc*.
[in left *Lactobacillus bulgaricus* phase contrast (3600), in right *Leuconostoc mesenteroides* phase-contrast micrograph].

3.2.3. Family *Enterococcaceae* and *Streptococcaceae*

Enterococcaceae (Enterococcus) and *Streptococcaceae (Streptococcus, Lactococcus)* are important families of chemoheterotrophic, mesophilic, nonsporing, Gram-positive cocci. In practice, they are often distinguished primarily based on phenotypic properties such as oxygen relationships, cell arrangement, the presence of catalase and cytochromes, and peptidoglycan structure.

The most important of these genera is *Streptococcus*, which is facultatively anaerobic and catalase negative. The streptococci and their close relatives, the enterococci and lactococci, occur in pairs or chains when grown in liquid media, do not form endospores, and usually are nonmotile. They all ferment sugars with lactic acid, but no gas, as the major product (homolactic fermentation). A few species are anaerobic rather than facultative. Many bacteria originally placed within the genus have been moved to two other genera, *Enterococcus* and *Lactococcus*. Many characteristics

are used to identify these cocci. One of their most important taxonomic characteristics is the ability to lyse erythrocytes when growing on blood agar, an agar medium containing 5% sheep or horse blood (α-hemolysis and β-hemolysis).

Pyogenic streptococci usually are pathogens and associated with pus formation (pyogenic means pus producing). Most species produce β-hemolysis (*S. pyogenes*) on blood agar and form chains of cells. The major human pathogen in this group is *S. pyogenes*, which causes streptococcal sore throat, acute glomerulonephritis, and rheumatic fever.

S. pneumoniae is α-hemolytic and grows as pairs of cocci. It is associated with lobar pneumonia and otitis media (inflammation of the middle ear).

S. mutans is associated with the formation of dental caries.

E. faecalis are normal residents of the intestinal tracts of humans and most other animals. *E. faecalis* is an opportunistic pathogen that can cause urinary tract infections and endocarditis.

L. lactis is widely used in the production of buttermilk and cheese because it can curdle milk and add flavor through the synthesis of diacetyl and other products.

Examples of classification:

Domaine:	Bacteria	Bacteria	Bacteria
Phylum:	Firmicutes	Firmicutes	Firmicutes
Class:	Clostridia	Bacilli	Bacilli
Order:	Clostridiales	Bacillales	Lactobacillales
Family:	Bacillaceae	Bacillaceae	Streptococcaceae
Genus:	Clostridium	Bacillus	Streptococcus
Species:	*Clostridium tetani*	*Bacillus subtilis*	*Streptococcus pneumoniae*