CHAPTER 06: PHYLUM PROTEOBACTERIA

1. Introduction

Volume 2 of the second edition of *Bergey's Manual* is devoted entirely to the **proteobacteria.** This is the largest and most diverse group of bacteria; currently there are over 500 genera. Although 16S rRNA studies show that they are phylogenetically related, proteobacteria vary markedly in many respects. The morphology of these Gram-negative bacteria ranges from simple rods and cocci to genera with prosthecae, buds, and even fruiting bodies. Physiologically they are just as diverse. Photoautotrophs, chemolithotrophs, and chemoheterotrophs are all well represented.

Comparison of 16S rRNA sequences has revealed five lineages of descent within the phylum *Proteobacteria*: *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, and *Epsilonproteobacteria* (**Fig. 01**).

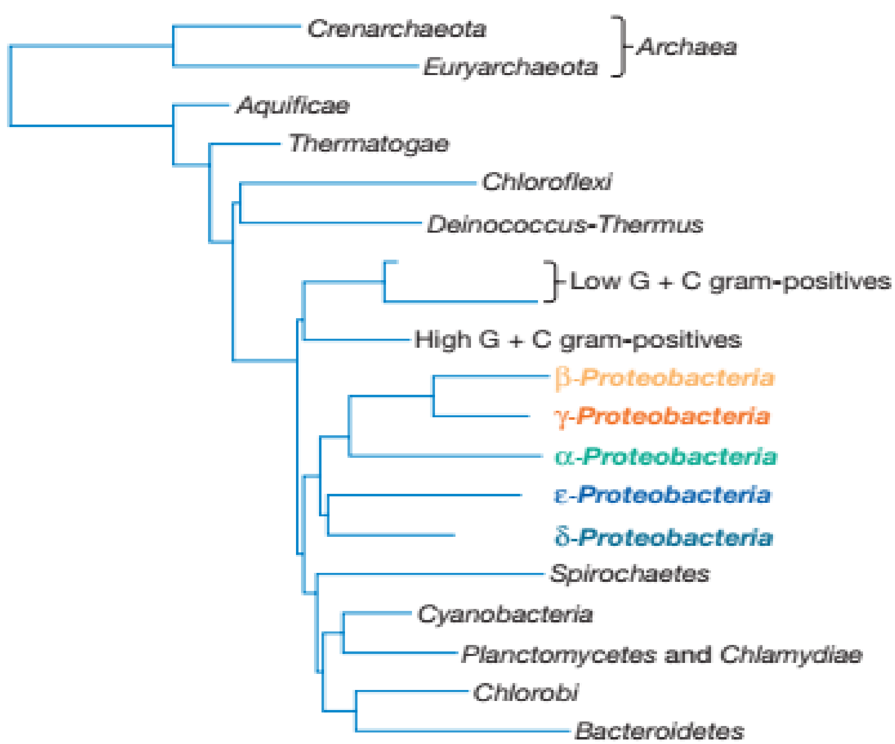


Figure 01: Phylogenetic relationships among the Procaryotes.
(The *Proteobacteria* are highlighted)

2. Class *Alphaproteobacteria*

The α-**proteobacteria** include most of the oligotrophic proteobacteria (those capable of growing at low nutrient levels). Some have unusual metabolic modes such as methylotrophy (*Methylobacterium*), chemolithotrophy (*Nitrobacter*), and the ability to fix nitrogen (*Rhizobium*). Members of genera such as *Rickettsia* and *Brucella* are important pathogens; in fact, *Rickettsia* is an obligate intracellular parasite. Many genera are characterized by distinctive morphology such as prosthecae. The class *Alphaproteobacteria* has seven orders and 20 families.

2.1. Purple nonsulfur bacteria

All the purple bacteria use anoxygenic photosynthesis, e.g. *Rhodospirillum* and *Azospirillum* (both in the family *Rhodospirillaceae*).

2.2. *Rickettsia*

Obligate intracellular parasites

2.3. *Caulobacteraceae* and *Hyphomicrobiaceae*

A number of the proteobacteria are not simple rods or cocci but have some sort of appendage. These bacteria have interesting life cycles that feature a prostheca or reproduction by **budding**. A **prostheca** (pl., prosthecae), also called a **stalk,** is an extension of the cell, including the plasma membrane and cell wall, that is narrower than the mature cell. The bud first appears as a small protrusion at a single point and enlarges to form a mature cell. The families *Hyphomicrobiaceae* (order *Rhizobiales*) and *Caulobacteraceae* of the α-proteobacteria contain two of the best studied prosthecate genera: *Hyphomicrobium* and *Caulobacter.*

The genus *Hyphomicrobium* contains chemoheterotrophic, aerobic, budding bacteria that frequently attach to solid objects in freshwater, marine, and terrestrial environments (**Fig. 02**). At the beginning of the reproductive cycle, the mature cell produces a prostheca (also called a **hypha**), that grows to several mm in length (**Fig. 02**). The nucleoid divides, and a copy moves into the hypha while a bud forms at its end. As the bud matures, it produces one to three flagella, and a septum divides the bud from the hypha. The bud is finally released as an oval- to pear-shaped swarmer cell, which swims off, then settles down and begins budding. The mother cell may bud several times at the tip of its hypha.

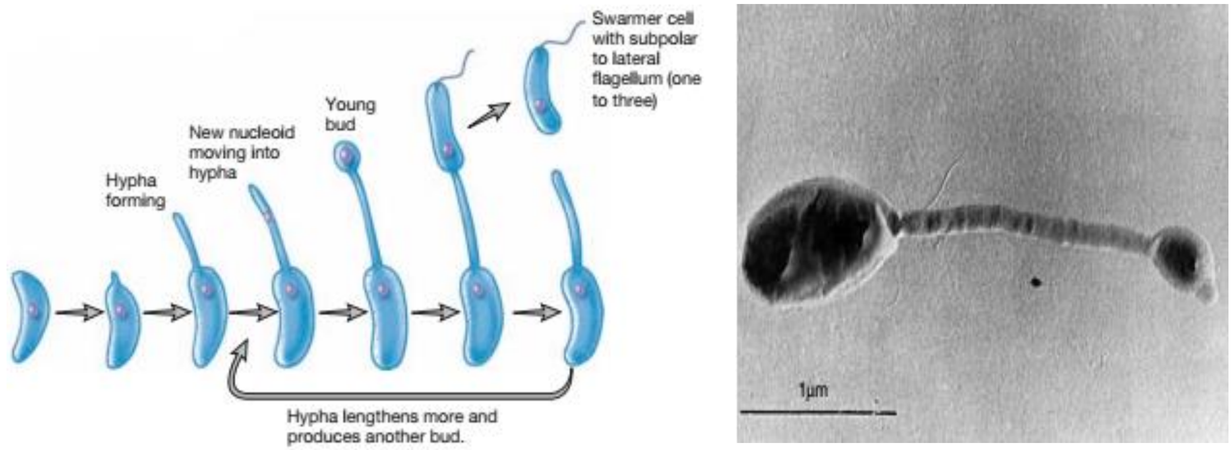
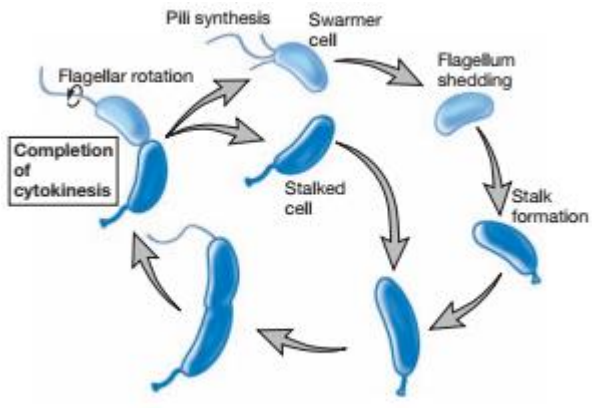


Figure 02: Life cycle of *Hyphomicrobium* (left) and Prosthecate, budding bacteria (right) (*Hyphomicrobium facilis* with hypha and young bud).

Hyphomicrobium grows on ethanol and acetate and flourishes with one-carbon compounds such as methanol, formate, and formaldehyde (sugars and most amino acids do not support abundant growth). That is, it is a facultative **methylotroph** and can derive both energy and carbon from reduced one carbon compounds.

Bacteria in the genus *Caulobacter* alternate between polarly flagellated rods and cells that possess a prostheca and **holdfast,** by which they attach to solid substrata (**Fig. 03**). Incredibly, the material secreted at the end of the *Caulobacter cresentus* holdfast is the strongest biological adhesion molecule known (a bacterial superglue). The prostheca differs from that of *Hyphomicrobium* in that it lacks cytoplasmic components and is composed almost totally of the plasma membrane and cell wall. It grows longer in nutrient-poor media and can reach more than 10 times the length of the cell body. The prostheca may improve the efficiency of nutrient uptake from dilute habitats by increasing surface area; it also gives the cell extra buoyancy.

The life cycle of *Caulobacter* is unusual (**Fig. 03**). When ready to reproduce, the cell elongates and a single polar flagellum forms at the end opposite the prostheca. The cell then undergoes asymmetric transverse binary fission to produce a flagellated swarmer cell that swims away. The swarmer, which cannot reproduce, comes to rest, ejects its flagellum, and forms a new prostheca on the formerly flagellated end. The new stalked cell then starts the cycle anew. This process takes about two hours to complete. The species *C. cresentus* has become an important model organism in the study of microbial development and the bacterial cell cycle.



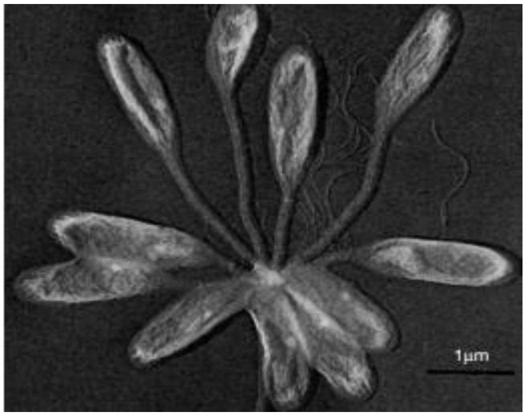


Figure 03: Caulobacter life cycle (left) and *Caulobacter*; a rosette of cells adhering to each other by their prosthecae (right).

[Stalked cells attached to a substrate undergo asymmetric binary fission producing a stalked and a flagellated cell, called a swarmer cell. The swarmer cell swims freely and makes pili until it settles, ejects its flagella, and forms a stalk. Only stalked cells can divide].

2.4. Family *Rhizobiaceae*

The important family in the order *Rhizobiales* is *Rhizobiaceae,* which includes the aerobic genera *Rhizobium* and *Agrobacterium.*

Members of the genus *Rhizobium* are motile rods that become pleomorphic under adverse conditions (**Fig. 04**). Cells often contain poly-β-hydroxybutyrate inclusions. They grow symbiotically within root nodule cells of legumes as nitrogen-fixing bacteroids. The proliferation of the *Leguminosae* (over 18,000 species) reflects their capacity to establish symbiotic relationships with bacteria that form nodules on their roots. Within the nodules the microbes reduce or fix atmospheric nitrogen into ammonium, making it directly available to the plant host.

The genus *Agrobacterium* is placed in the family *Rhizobiaceae* but differs from *Rhizobium* in not stimulating root nodule formation or fixing nitrogen. Instead agrobacteria invade the crown, roots, and stems of many plants and transform plant cells into autonomously proliferating tumor cells. Most of the genes that encode distinguishing characteristics are carried on plasmids (*see figure 29.19*). The best-studied species is *A. tumefaciens,* which enters many broad-leaved plants through wounds and causes crown gall disease (**Fig. 04**). The ability to produce tumors depends on the presence of a large Ti (for *t*umor*i*nducing) plasmid.

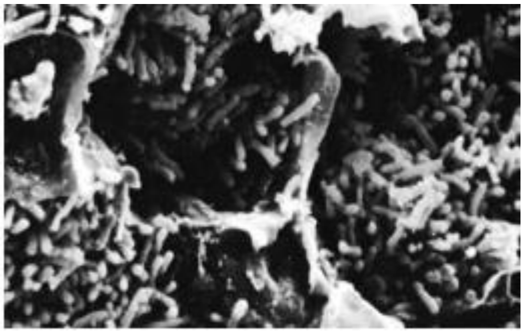


Figure 04: *Rhizobium* (left) and *Agrobacterium* (right).
[*Rhizobium leguminosarum* with two polar flagella (314,000); Crown gall tumor of a tomato plant caused by *Agrobacterium tumefaciens*].

2.5. Nitrifying bacteria

The **nitrifying bacteria** are a very diverse collection of bacteria. *Bergey's Manual* places nitrifying genera in three classes (α-, β-, and γ-proteobacteria) and several families: *Nitrobacter* in the *Bradyrhizobiaceae*, α-proteobacteria; *Nitrosomonas* and *Nitrosospira* in the *Nitrosomonadaceae,* γ-proteobacteria; *Nitrococcus* in the *Ectothiorhodospiraceae,* γ-proteobacteria; and *Nitrosococcus* in the *Chromatiaceae,* γ-proteobacteria. All are aerobic, Gram-negative organisms with the ability to capture energy from the oxidation of either ammonia or nitrite. Nitrifiers may be rod-shaped, ellipsoidal, spherical, spirillar or lobate, and they may possess either polar or peritrichous flagella. Identification is based on properties such as their preference for nitrite or ammonia, their general shape, and the nature of any cytomembranes present.

Nitrifying bacteria make important contributions to the nitrogen cycle. In soil, sewage disposal systems, and freshwater and marine habitats, the β-proteobacteria *Nitrosomonas* and *Nitrospira* and the γ-proteobacterium *Nitrosococcus* oxidize ammonia to nitrite. In the same niches, members of the γ-proteobacterial genus *Nitrococcus* then oxidize nitrite to nitrate. The whole process of converting ammonia to nitrite to nitrate is called **nitrification** and it occurs rapidly in oxic soil treated with fertilizers containing ammonium salts. Nitrate is readily used by plants.

3. Class *Betaproteobacteria*

The β**-proteobacteria** overlap the α-proteobacteria metabolically but tend to use substances that diffuse from organic decomposition in the anoxic zone of habitats. Some of these bacteria use hydrogen ammonia, methane, volatile fatty acids, and similar substances. The β-proteobacteria may be chemoheterotrophs, photolithotrophs, methylotrophs, and chemolithotrophs.

The class *Betaproteobacteria* has seven orders and 12 families. Two genera are important human pathogens: *Neisseria* and *Bordetella*.

3.1. Order *Neisseriales*

The order *Neisseriales* has one family, *Neisseriaceae*, with 15 genera, type genus is *Neisseria*. Members of this genus are nonmotile, aerobic, Gram-negative cocci that most often occur in pairs with adjacent sides flattened. They may have capsules and fimbriae. The genus is chemoorganotrophic, oxidase positive and catalase positive. They are inhabitants of the mucous membranes of mammals, and some are human pathogens. *Neisseria gonorrhoeae* is the causative agent of gonorrhea; *Neisseria meningitidis* is responsible for some cases of bacterial meningitis.

3.2. Order *Burkholderiales*

The order contains four families, the family *Alcaligenaceae* contains the genus *Bordetella*. This genus is composed of gram-negative, aerobic coccobacilli, about 0.2 to 0.5 by 0.5 to 2.0 mm in size. *Bordetella* is a chemoorganotroph with respiratory metabolism that requires organic sulfur and nitrogen (amino acids) for growth. It is a mammalian parasite that multiplies in respiratory epithelial cells. *Bordetella pertussis* is a nonmotile, encapsulated species that causes whooping cough.

Some genera in the order have a **sheath**—a hollow, tubelike structure surrounding a chain of cells. Sheaths help bacteria attach to solid surfaces and acquire nutrients from slowly running water as it flows past, even if it is nutrient-poor. Sheaths also protect against predators such as protozoa. Two well-studied sheathed genera are *Sphaerotilus* and *Leptothrix*.

3.3. Order *Nitrosomonadales*

A number of chemolithotrophs are found in the order *Nitrosomonadales*. Two genera of nitrifying bacteria (*Nitrosomonas* and *Nitrosospira*) are members of the family *Nitrosomonadaceae*. The stalked chemolithotroph *Gallionella* is in this order. The family *Spirillaceae* has one genus, *Spirillum* (**Fig. 05**).

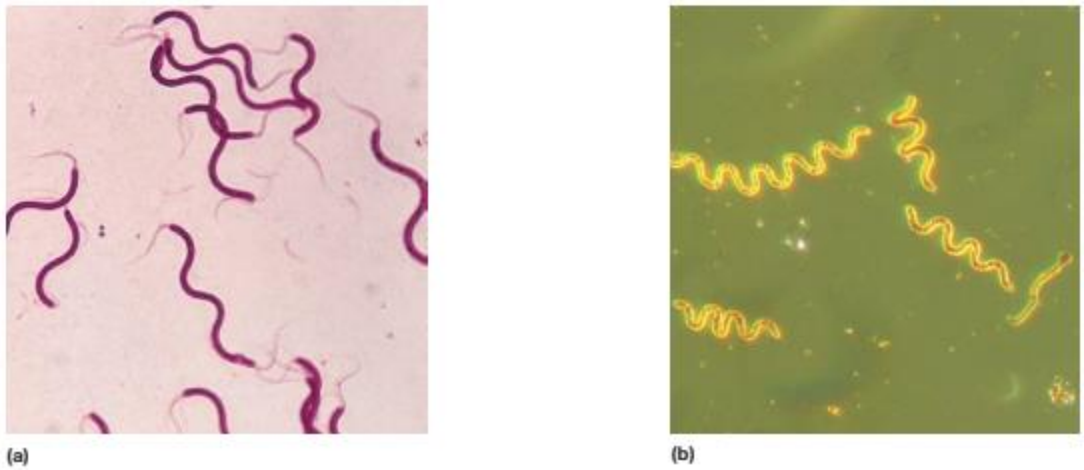


Figure 05: Genus *Spirillum.*
[(a) *Spirillum volutans* with bipolar flagella visible (3450). (b) *Spirillum volutans*; phase contrast (3550)].

4. Class *Gammaproteobacteria*

The γ**-proteobacteria** constitute the largest subgroup of proteobacteria with an extraordinary variety of physiological types. Many important genera are chemoorganotrophic and facultatively anaerobic. Other genera contain aerobic chemoorganotrophs, photolithotrophs, chemolithotrophs, or methylotrophs. The exceptional diversity of these bacteria is evident from the fact that *Bergey's Manual* divides the class *Gammaproteobacteria* into 14 orders and 28 families.

4.1. The Purple Sulfur Bacteria

As mentioned previously, the purple photosynthetic bacteria are distributed between three subgroups of the proteobacteria. Most of the purple nonsulfur bacteria are α-proteobacteria but the purple sulfur bacteria are γ-proteobacteria. *Bergey's Manual* divides the purple sulfur bacteria into two families: the *Chromatiaceae* and *Ectothiorhodospiraceae* in the order *Chromatiales.*

The **purple sulfur bacteria** are strict anaerobes and usually photolithoautotrophs. *Thiospirillum*, *Thiocapsa*, and *Chromatium* are typical purple sulfur bacteria. They are found in anoxic, sulfide-rich zones of lakes, bogs, and lagoons where large blooms can occur under certain conditions.

4.2. Order *Thiotrichales*

The order *Thiotrichales* contains three families, the largest of which is the family *Thiotrichaceae.* This family has several genera that oxidize sulfur compounds. Morphologically both rods and filamentous forms are present. Two of the best-studied gliding genera in this family are *Beggiatoa* and *Leucothrix*.

Beggiatoa is microaerophilic, filaments form and grows in sulfide-rich habitats such as sulfur springs, freshwater with decaying plant material, rice paddies, salt marshes, and marine sediments. It oxidizes hydrogen sulfide to form large sulfur grains located in pockets formed by invaginations of the plasma membrane. *Beggiatoa* can subsequently oxidize the sulfur to sulfate.

Leucothrix is an aerobic chemoorganotroph that forms filaments and dispersed by the formation of gonidia. It is usually marine and is attached to solid substrates by a holdfast.

4.3. Order *Pseudomonadales*

Pseudomonas is the most important genus in the order *Pseudomonadales*, the family *Pseudomonaceae*. These bacteria are straight or slightly curved rods, and are motile by one or several polar flagella (**Fig. 06**). These chemoheterotrophs usually carry out aerobic respiration. Sometimes nitrate is used as the terminal electron acceptor in anaerobic respiration. All pseudomonads have a functional tricarboxylic acid cycle and can oxidize substrates completely to CO_2.

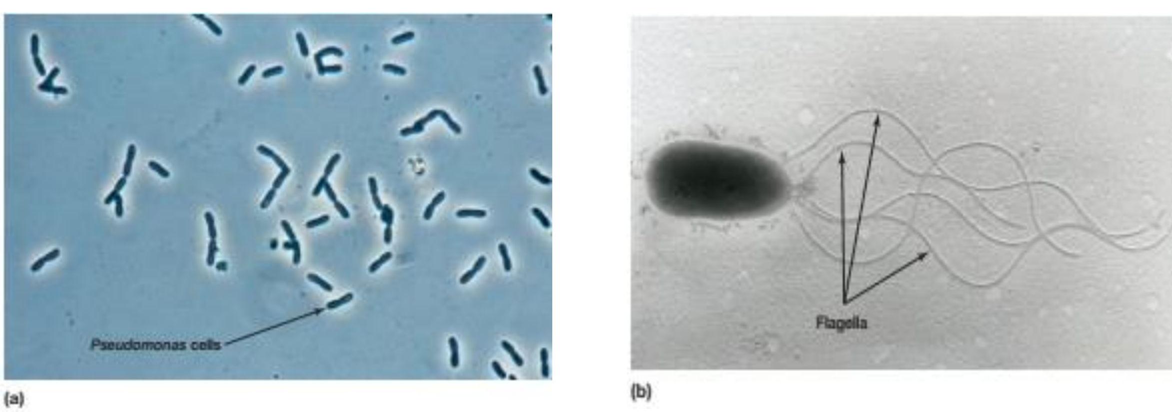


Figure 06: Genus *Pseudomonas*.
[(a) A phase-contrast micrograph of *Pseudomonas* cells containing PHB (poly-β-hydroxybutyrate) granules. (b) A transmission electron micrograph of *Pseudomonas putida* with five polar flagella, each flagellum about 5–7 mm in length].

The genus *Pseudomonas* is an exceptionally heterogeneous taxon currently composed of about 60 species. The three best characterized groups are subdivided according to properties such as the presence of poly-β-hydroxybutyrate (PHB), the production of a fluorescent pigment, pathogenicity, the presence of arginine dihydrolase, and glucose utilization. For example, the fluorescent subgroup does not accumulate PHB and produces a diffusible, water-soluble, yellow-green pigment that fluoresces under UV radiation. *Pseudomonas aeruginosa, P. fluorescens, P. putida*, and *P. syringae* are members of this group.

The pseudomonads have a great practical impact in several ways, including these:

1. Many can degrade an exceptionally wide variety of organic molecules. Thus, they are very important in the **mineralization** process (the microbial breakdown of organic materials to inorganic substances) in nature and in sewage treatment.

2. Several species (e.g., *P. aeruginosa*) are important experimental subjects. For example, the study of *P. aeruginosa* has significantly advanced our understanding of how bacteria form biofilms and the role of extracellular signaling in bacterial communities and pathogenesis. The genome of *P. aeruginosa* has an unusually large number of genes for catabolism, nutrient transport, the efflux of organic molecules, and metabolic regulation. This may explain its ability to grow in many environments and resist antibiotics.

3. Some pseudomonads are major animal and plant pathogens. *P. aeruginosa* infects people with low resistance such as cystic fibrosis patients. It also invades burns, and causes urinary tract infections. *P. syringae* is an important plant pathogen.

4. Pseudomonads such as *P. fluorescens* are involved in the spoilage of refrigerated milk, meat, eggs, and seafood because they grow at 4°C and degrade lipids and proteins.

The genus *Azotobacter* also is in the family *Pseudomonadaceae.* The genus contains large, ovoid bacteria, that may be motile by peritrichous flagella. The cells are often pleomorphic, ranging from rods to coccoid shapes, and form cysts as the culture ages. The genus is aerobic, catalase positive, and fixes nitrogen nonsymbiotically. *Azotobacter* is widespread in soil and water.

4.4. Order *Enterobacteriales*

The family *Enterobacteriaceae* is the largest of the family, it contains Gram-negative, peritrichously flagellated or nonmotile, facultatively anaerobic, straight rods with simple nutritional requirements. The order *Enterobacteriales* has only one family, *Enterobacteriaceae*, with 44 genera.

The metabolic properties of the *Enterobacteriaceae* are very useful in characterizing its constituent genera. Members of the family, often called **enterobacteria** or **enteric bacteria** [Greek *enterikos*, pertaining to the intestine], all degrade sugars by means of the Embden-Meyerhof pathway and cleave pyruvic acid to yield formic acid in formic acid fermentations. Those enteric bacteria that produce large amounts of gas during sugar fermentation, such as *Escherichia* spp., have the formic hydrogenlyase complex that degrades formic acid to H2 and CO2.

Because the enteric bacteria are so similar in morphology, biochemical tests are normally used to identify them after a preliminary examination of their morphology, motility, and growth responses **Table 01** summarizes a few of the biochemical properties useful in distinguishing between genera of enteric bacteria. The usefulness of biochemical tests in identifying enteric bacteria is shown by the popularity of commercial identification systems, such as the Enterotube and API 20-E systems, that are based on these tests.

Table 01: Some Characteristics of Selected Genera in the *Enterobacteriaceae*

Characteristics	*Escherichia*	*Shigella*	*Salmonella*	*Citrobacter*	*Proteus*
Methyl red	+	+	+	+	+
Voges-Proskauer	−	−	−	−	d
Indole production	(+)	d	−	d	d
Citrate use	−	−	(+)	+	d
H_2S production	−	−	(+)	d	(+)
Urease	−	−	−	(+)	+
β-galactosidase	(+)	d	d	+	−
Gas from glucose	+	−	(+)	+	+
Acid from lactose	+	−	(−)	d	−
Phenylalanine deaminase	−	−	−	−	+
Lysine decarboxy-lase	(+)	−	(+)	−	−
Ornithine decarboxylase	(+)	d	(+)	(+)	d
Motility	d	−	(+)	+	+
Gelatin liquifaction (22°C)	−	−	−	−	+
% G + C	48–59	49–53	50–53	50–52	38–41
Genome size (Mb)	4.6–5.5	4.6	4.5–4.9	Nd[d]	Nd
Other characteristics	1.1–1.5 × 2.0–6.0 μm; peritrichous when motile	No gas from sugars	0.7–1.5 × 2–5 μm; peritrichous flagella	1.0 × 2.0–6.0 μm; peritrichous	0.4–0.8 × 1.0–3.0 μm; peritrichous

[a](1) usually present;
[b](2) usually absent;
[c]d, strains or species vary in possession of characteristic;
[d]Nd: Not determined; genome not yet sequenced.

Members of the *Enterobacteriaceae* are so common, widespread, and important that they are probably more often seen in most laboratories than any other bacteria. *Escherichia coli* is undoubtedly the best-studied bacterium and the experimental organism of choice for many microbiologists. It is an inhabitant of the colon of humans and other warm-blooded animals, and it is quite useful in the analysis of water for fecal contamination. Some strains cause gastroenteritis or urinary tract infections. Several genera contain very important human pathogens responsible for a variety of diseases: *Salmonella*, typhoid fever and gastroenteritis; *Shigella*, bacillary dysentery; *Klebsiella*, pneumonia; *Yersinia*, plague. Members of the genus *Erwinia* are major pathogens of crop plants and cause blights, wilts, and several other plant diseases.

4.5. Order *Vibrionales*

The order *Vibrionales* contains only one family, the *Vibrionaceae*. Members of the family *Vibrionaceae* are Gram-negative, straight or curved rods with polar flagella. Most are oxidase positive, and all use D-glucose as their sole or primary carbon and energy source. The majority are aquatic microorganisms, widespread in freshwater and the sea.

Several vibrios are important pathogens. *Vibrio cholerae* causes cholera, and *V. parahaemolyticus* can cause gastroenteritis in humans following consumption of contaminated seafood. *V. anguillarum* and others are responsible for fish diseases.

4.6. Order *Pasteurellales*

The members of the family *Pasteurellaceae* are small and nonmotile, normally oxidase positive, have complex nutritional requirements of various kinds, and are parasitic in vertebrates. Type genus is *Pasteurella*.

As might be expected, members of this family are best known for the diseases they cause in humans and many animals. *Pasteurella multocida* and *P. haemolytica* are important animal pathogens. *P. multocida* is responsible for fowl cholera, which kills many chickens, turkeys, ducks, and geese each year. *P. haemolytica* is at least partly responsible for pneumonia in cattle, sheep, and goats (e.g., “shipping fever” in cattle). *Haemophilus influenzae* type b is a major human pathogen that causes a variety of diseases, including meningitis in children.