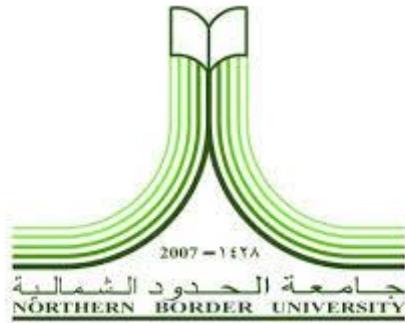


Kingdom of Saudi Arabia
Ministry of Higher Education
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Bacteriology II

3303-314

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The Phylogeny of *Bacteria*

In this course Bacteriology II we expand the evolutionary relationships among microorganisms with a consideration of the properties and diversity of major microbial groups. We begin our tour with a focus on *Proteobacteria*, a major group within Bacteria that contains many of the most commonly encountered bacteria.

With several thousand species of bacteria described, we obviously cannot consider them all. Therefore, using a phylogenetic tree to focus our discussion, we will explore some of the best known species, particularly ones for which much phenotypic information is available. For more detailed information on prokaryotic diversity the reader is directed to the two major reference sources: *Bergey's Manual of Systematic Bacteriology* and *The Prokaryotes*.

1.1 Phylogenetic Overview of *Bacteria*

Many major lineages, called *phyla*, of Bacteria are known from the study of laboratory cultures, and many others have been identified from the retrieval and sequencing of ribosomal RNA (rRNA) genes from microbial communities in natural habitats. **Figure 1** gives an overview of the phylogeny of major *phyla* of Bacteria for which laboratory cultures have been obtained. When one includes *phyla* of Bacteria known only from 16S rRNA sequences retrieved from the environment, well over 80 *phyla* can be distinguished. However, since little phenotypic information is available on species in which cultures have not yet been obtained, we focus here on *phyla* with cultured species. As Figure 1 clearly shows, the most phylogenetically ancient (least derived) phylum contains the genus *Aquifex* and relatives, all of which are *hyperthermophilic* H₂-oxidizing *chemolithotrophs*. Other “early” *phyla* such as *Thermodesulfobacterium*,

Thermotoga, and the *Chloroflexus* group (green nonsulfur bacteria) also contain *thermophilic* species.

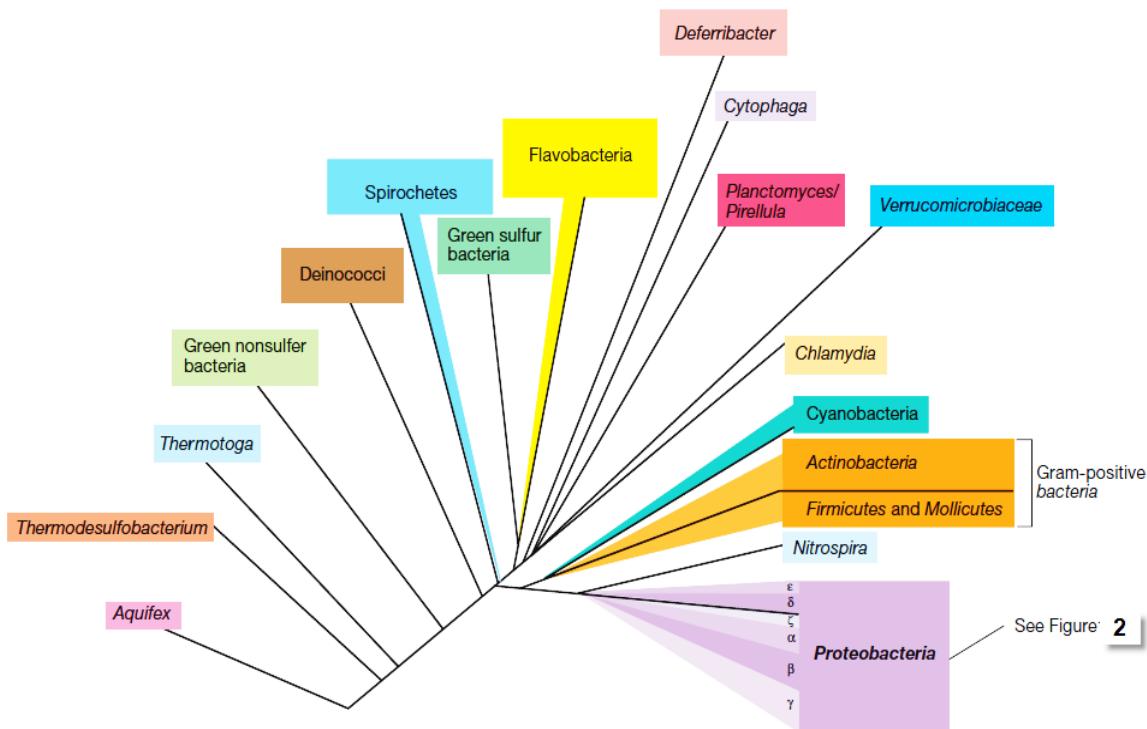


Figure 1: Some major *phyla* of *Bacteria* based on 16S ribosomal RNA gene sequence comparisons. Over 80 *phyla* of *Bacteria* are currently known, including many *phyla* known only from environmental sequences obtained in community sampling.

Continuing past the green nonsulfur bacteria, we see the *deinococci* and relatives, the morphologically unique *spirochetes*, the phototrophic green sulfur bacteria, the *chemoorganotrophic* *Flavobacterium* and *Cytophaga* groups, the budding *Planctomyces–Pirellula* and the *Verrucomicrobium* groups, the *Chlamydia*, and the genera *Nitrospira* and *Deferrribacter* (Figure 1). Other major groups include the gram-positive bacteria and the *cyanobacteria*. The gram-positive bacteria are a large group of primarily *chemoorganotrophic* Bacteria. They can be separated into two subgroups called the *Firmicutes* and the *Actinobacteria*. The *cyanobacteria* are oxygenic *phototrophic* bacteria with evolutionary roots near those of the gram-positive Bacteria. The remaining phylum of cultured Bacteria,

the *Proteobacteria* (Figure 2), is by far the largest and most metabolically diverse of all Bacteria. *Proteobacteria* constitute the majority of known bacteria of medical, industrial, and agricultural significance. As a group, the *Proteobacteria* are all gram-negative bacteria. They show an exceptionally wide diversity of energy-generating mechanisms, with *chemolithotrophic*, *chemoorganotrophic*, and *phototrophic* species (Figure 2).

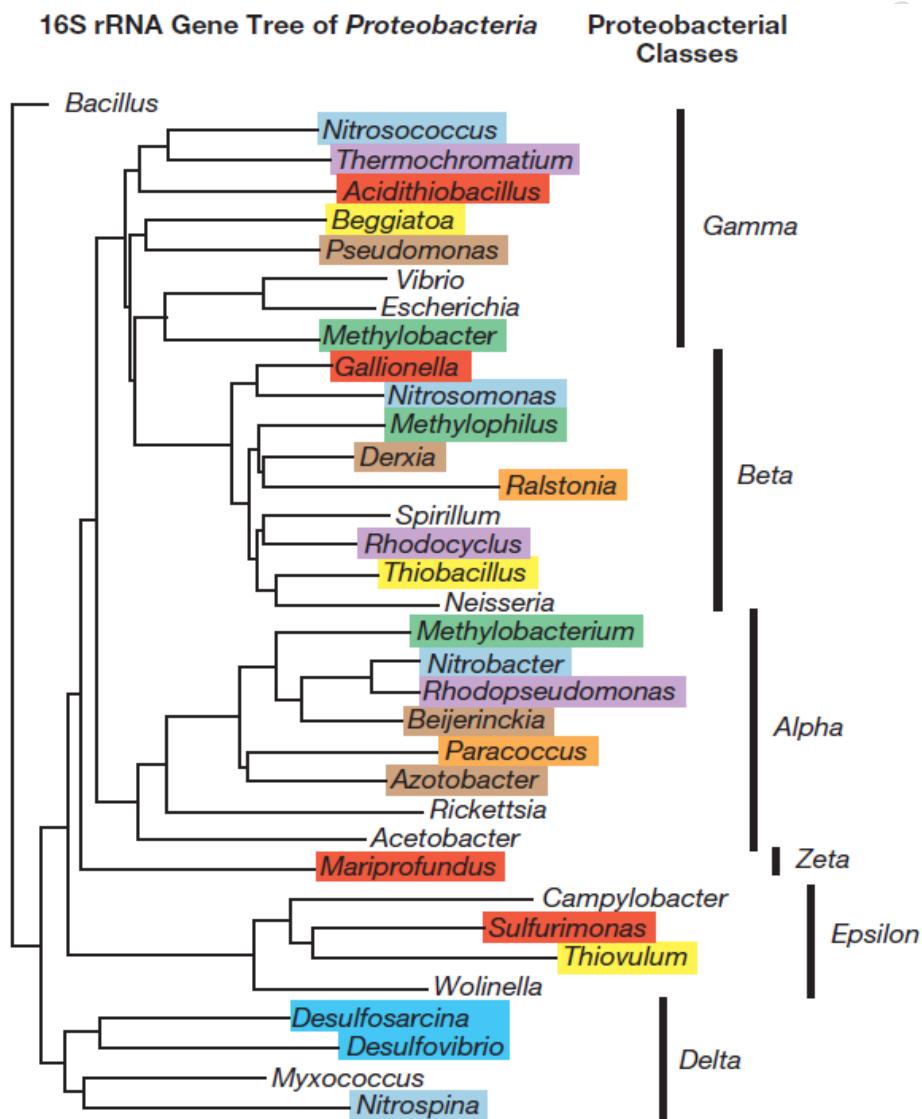


Figure 2: Phylogenetic tree of some key genera of *Proteobacteria*. The tree was constructed by comparative 16S rRNA sequencing. Note how identical metabolisms are often distributed in phylogenetically distinct genera, suggesting that horizontal gene flow has been extensive in the *Proteobacteria*. Some organisms listed may have multiple properties; for example, some sulfur *chemolithotrophs* are also iron or hydrogen *chemolithotrophs*, and several of the organisms listed can fix nitrogen. Phylogenetic analyses were performed and the phylogenetic tree constructed by Marie Asao.

The *Proteobacteria* are equally diverse in terms of their relationship to oxygen (O_2), with *anaerobic*, *microaerophilic*, and *facultatively aerobic* species known. Morphologically, they also exhibit a wide range of cell shapes, including straight and curved *rods*, *cocci*, *spirilla*, *filamentous*, *budding*, and *appendaged* forms.

Based on 16S rRNA gene sequences, the phylum *Proteobacteria* can be divided into six classes, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, and *Zetaproteobacteria*, each containing many genera and species. The *Zeta* class is currently composed of only one organism, the marine iron-oxidizing bacterium *Mariprofundus*, but other relatives almost certainly exist. Despite the phylogenetic breadth of the *Proteobacteria*, species in different classes often have similar or even identical metabolisms. For example, *phototrophy* and *methylotrophy* occur in species of three different classes of *Proteobacteria*, and ammonia and nitrite-oxidizing (*nitrifying*) bacteria span four different classes of *Proteobacteria* (Figure 2) plus an additional genus that forms the heart of a separate phylum of Bacteria! This observation strongly suggests that gene sharing by horizontal gene flow has played a major role in shaping the metabolic diversity of the *Proteobacteria*. The sharing of metabolic traits in the different classes of *Proteobacteria* is also a good reminder that phenotype and phylogeny often give different views of prokaryotic diversity. We now consider the major groups of *Proteobacteria*, grouping them along some common phenotypic themes.

II- Phototrophic, Chemolithotrophic, and Methanotrophic *Proteobacteria*

The first groups of *Proteobacteria* we consider are those able to carry out *anoxygenic* photosynthesis, or sulfur-, iron-, hydrogen-, or nitrogen-dependent

chemolithotrophy. We will also consider bacteria that oxidize methane (CH_4). We begin with purple bacteria, classic examples of the *phototrophic* lifestyle.

1.2 Purple Phototrophic Bacteria

Key Genera: *Chromatium*, *Ectothiorhodospira*, *Rhodobacter*, *Rhodospirillum*.

The purple phototrophic bacteria carry out *anoxygenic photosynthesis*. Thus, unlike the *cyanobacteria*, which are *oxygenic* phototrophs, no O_2 is released. Purple bacteria are a morphologically diverse group, and the classification of these organisms has been established along phylogenetic, morphological, and physiological lines. Different genera fall within the *Alpha*-, *Beta*-, or *Gamma-proteobacteria* (see Tables 1 and 2). Purple bacteria contain bacteriochlorophylls and carotenoid pigments. Together, these pigments give purple bacteria their spectacular colors, usually purple, red, or orange. Purple bacteria produce intracytoplasmic photosynthetic membrane systems into which their pigments are inserted. These membranes can be of various arrangements (Figure 3) but in all cases originate from invaginations of the cytoplasmic membrane.

Table 1 Genera and characteristics of purple sulfur bacteria^a

Characteristics	Genus
Sulfur deposited externally	
Spirilla, polar flagella	<i>Ectothiorhodospira</i>
Spirilla, extreme alkaliphiles	<i>Thiorhodospira</i>
Spirilla, extreme halophiles	<i>Halorhodospira</i>
Sulfur deposited internally	
Do not contain gas vesicles	
Ovals or rods, polar flagella	<i>Chromatium</i> <i>Allochromatium</i> <i>Halochromatium</i> <i>Rhabdochromatium</i> <i>Thermochromatium</i> <i>Isochromatium</i> <i>Marichromatium</i>
Spheres, alkaliphilic	<i>Thioalkalococcus</i>
Spheres, contain bacteriochlorophyll b	<i>Thioflavicoccus</i>
Spheres, diplococci, tetrads, nonmotile; cells 1.2–3 μm in diameter	<i>Thiocapsa</i>
Spheres or ovals, polar flagella; cells 2.5–3 μm in diameter	<i>Thiocystis</i>
Spheres, 1.5–2.5 μm in diameter	<i>Thiohalocapsa</i>
Spheres, 1–2 μm in diameter	<i>Thiorhodococcus</i>
Spheres, 1.2–1.5 μm in diameter	<i>Thiococcus</i>
Large spirilla, polar flagella	<i>Thiospirillum</i>
Small spirilla	<i>Thiorhodovibrio</i>
Contain gas vesicles	
Irregular spheres forming platelets of 4–16 cells	<i>Thiolampruvum</i>
Rods	<i>Lamprobacter</i>
Spheres, ovals, polar flagella	<i>Lamprocystis</i>
Rods, nonmotile; forming irregular network	<i>Thiodictyon</i>
Spheres, nonmotile; forming flat sheets of tetrads	<i>Thiopedia</i>

^aFrom a phylogenetic standpoint, all are species of *Gammaproteobacteria*.

These internal membranes allow purple bacteria to increase the amount of pigment they contain and to thus better utilize the available light. When cells are grown at high light intensities, photosynthetic membranes are few and pigment contents are low. By contrast, at low light intensities, the cells are packed with membranes and pigments.

Table 2 Genera and characteristics of purple nonsulfur bacteria^a

Characteristics	Genus	
Alphaproteobacteria		
Spirilla, polarly flagellated	<i>Rhodospirillum</i> <i>Phaeospirillum</i> <i>Rhodovibrio</i> <i>Rhodothalassium</i> <i>Roseospira</i> <i>Rhodospira</i> <i>Roseospirillum</i>	
Rods, polarly flagellated; divide by budding	<i>Rhodopseudomonas</i> <i>Rhodoplanes</i> <i>Rhodobium</i>	
Rods; divide by binary fission	<i>Rhodobacter</i>	
Ovoid to rod-shaped cells	<i>Rhodovulum</i>	
Ovals, peritrichously flagellated; growth by budding and hypha formation	<i>Rhodomicrobium</i>	
Large spheres, acidophilic (pH 5 optimum)	<i>Rhodopila</i>	
Small spheres, alkaliphilic (pH 9 optimum)	<i>Rhodobaca</i>	
Betaproteobacteria		
Ring-shaped or spirilla	<i>Rhodococcus</i>	
Curved rods	<i>Rubrivivax</i>	
Curved rods	<i>Rhodoferax</i>	

^aAll are members of the *Proteobacteria* (see Figure 17.2).

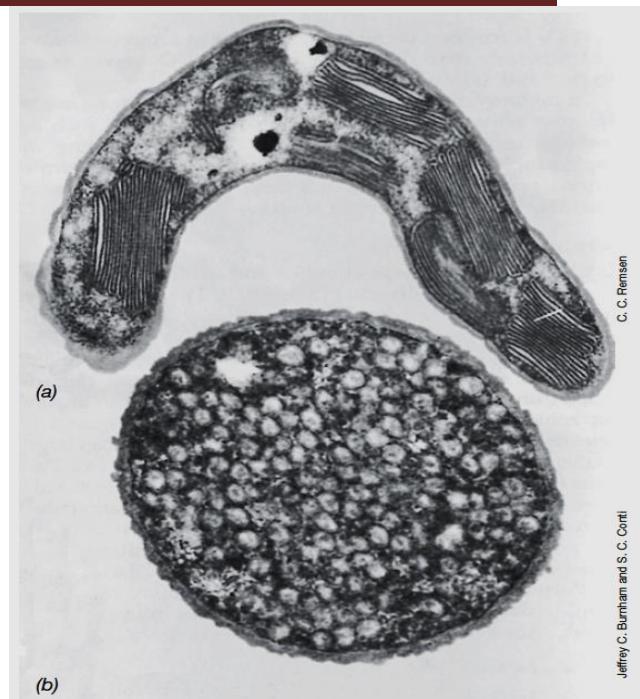


Figure 3: Membrane systems of phototrophic purple bacteria as revealed by the electron microscope. (a) *Ectothiorhodospira mobilis*, showing the photosynthetic membranes in flat sheets (lamellae). (b) *Allochromatium vinosum*, showing the membranes as individual, spherical vesicles.

Purple Sulfur Bacteria

Purple bacteria that utilize hydrogen sulfide (H_2S) as an electron donor for CO_2 reduction in photosynthesis are called **purple sulfur bacteria** (Table 1). The H_2S is oxidized to elemental sulfur (S^0) that is stored in globules inside the cells (Figure 4); the sulfur later disappears as it is oxidized to sulfate (SO_4^{2-}). Many purple sulfur bacteria can also use other reduced sulfur compounds as photosynthetic

electron donors; for example, thiosulfate ($S_2O_3^{2-}$) is commonly used to grow laboratory cultures. All purple sulfur bacteria discovered thus far are *Gammaproteobacteria* and use the Calvin cycle to support autotrophy.

Purple sulfur bacteria are generally found in illuminated *anoxic* zones of lakes and other aquatic habitats where H_2S accumulates, and also in “sulfur springs,” where geochemically or biologically produced H_2S can trigger the formation of mass developments of cells of purple sulfur bacteria (Figure 5). The most favorable lakes for development of purple sulfur bacteria are **meromictic** (permanently stratified) lakes. Meromictic lakes stratify because they have denser (usually saline) water in the bottom and less dense (usually freshwater) nearer the surface.

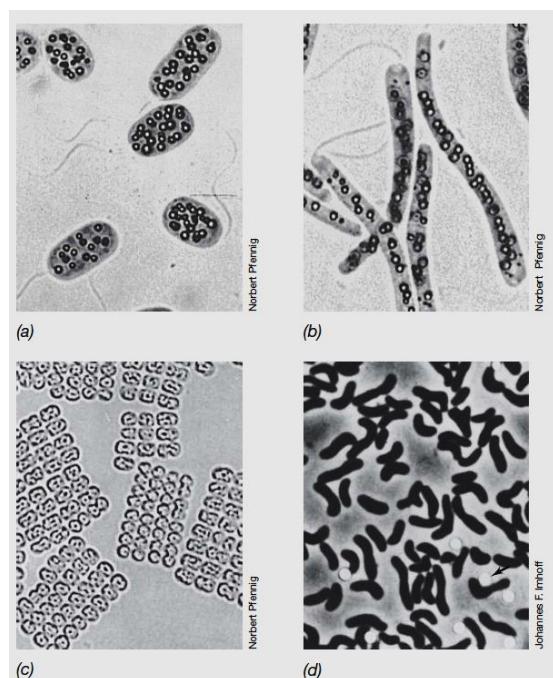


Figure 4: Bright-field and phase-contrast photomicrographs of purple sulfur bacteria. (a) *Chromatium okenii*; cells are about 5 μm wide. Note the globules of elemental sulfur inside the cells. (b) *Thiospirillum jenense*, a very large, polarly flagellated spiral; cells are about 30 μm long. Note the sulfur globules. (c) *Thiopedia rosea*; cells are about 1.5 μm wide. (d) Phase micrograph of cells of *Ectothiorhodospira mobilis*. Cells are about 0.8 μm wide. Note external sulfur globules (arrow).

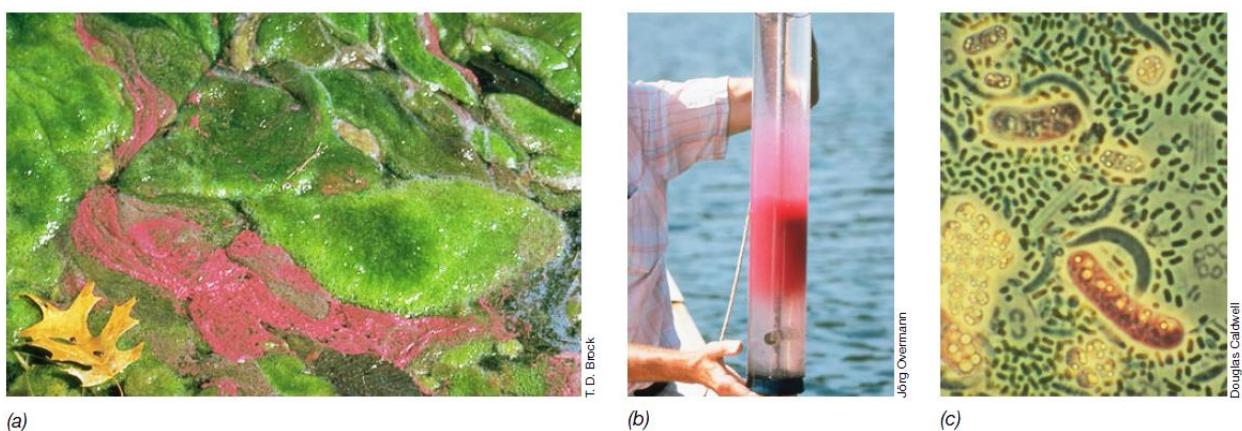


Figure 5: Blooms of purple sulfur bacteria. (a) *Lamprocystis roseopersicina*, in a sulfide spring. The bacteria grow near the bottom of the spring pool and float to the top (by virtue of their gas vesicles) when disturbed. The green color is from cells of the eukaryotic alga *Spirogyra*. (b) Sample of water from a depth of 7 m in Lake Mahoney, British Columbia. The major organism is *Amoeobacter purpureus*. (c) Phase-contrast photomicrograph of layers of purple sulfur bacteria from a small, stratified lake in Michigan. The purple sulfur bacteria include *Chromatium* species (large rods) and *Thiocystis* (small cocci).

If sufficient sulfate is present to support sulfate reduction, the sulfide, produced in the sediments, diffuses upward into the *anoxic* bottom waters, and here purple sulfur bacteria can form dense cell masses, called *blooms*, usually in association with green *phototrophic* bacteria (Figure 5c). Unlike other purple sulfur bacteria, the genera *Ectothiorhodospira* and *Halorhodospira* produce S⁰ (from the oxidation of H₂S) outside rather than inside the cell (Figure 4d). These genera are also interesting because many species are *extremely halophilic* (salt-loving) or *alkaliphilic* and are among the most extreme in these characteristics of all known Bacteria. These organisms are typically found in saline lakes, soda lakes, and salterns, where abundant levels of SO₄²⁻ support sulfate-reducing bacteria, the organisms that produce H₂S.

Purple Nonsulfur Bacteria

The term purple nonsulfur bacteria (PNSB) has been proposed for the physiological groups of *anaerobic phototrophic* *Alphaproteobacteria* and *Betaproteobacteria* that contain photosynthetic pigments and are able to perform *anoxygenic* photosynthesis. Some purple bacteria are called **purple nonsulfur bacteria** because it was originally thought that they were unable to use H₂S as an electron donor for the reduction of CO₂ to cell material. In fact, H₂S can be used by most species in this group, although the levels ideal for purple sulfur bacteria (1–3 mM) are typically toxic to most purple

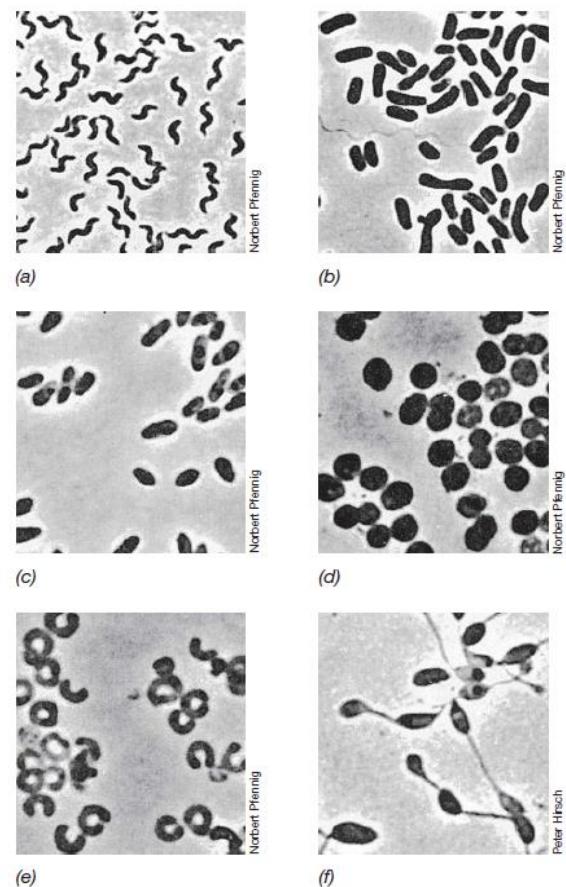


Figure 6: Representatives of several genera of purple nonsulfur bacteria. (a) *Phaeospirillum fulvum*; cells are about 3 μm long. (b) *Rhodoblastus acidophilus*; cells are about 4 μm long. (c) *Rhodobacter sphaeroides*; cells are about 1.5 μm wide. (d) *Rhodopila globiformis*; cells are about 1.6 μm wide. (e) *Rhodocyclus purpureus*; cells are about 0.7 μm in diameter. (f) *Rhodomicrobium vannielii*; cells are about 1.2 μm wide. See also Table 17.2.

nonsulfur bacteria. The morphological diversity of purple nonsulfur bacteria is as extensive as that of purple sulfur bacteria (**Table 2** and **Figure 6**), and all purple nonsulfur bacteria isolated thus far are either *Alpha-* or *Betaproteobacteria* (Table 2). Some purple nonsulfur bacteria can also grow *anaerobically* in the dark using fermentative or *anaerobic* respiratory metabolism, and most can grow aerobically in darkness by respiration. Under the latter conditions, synthesis of the photosynthetic machinery is repressed by O₂, and the electron donor can be an organic compound or in some species even an inorganic compound, such as H₂. However, it is the capacity of this group for *photoheterotrophy* (a condition where light is the energy source and an organic compound is the carbon source) that likely spells their competitive success in nature. Purple nonsulfur bacteria are typically nutritionally diverse, using fatty, organic, or amino acids; sugars; alcohols; or even aromatic compounds like benzoate or toluene as carbon sources. Most species can also grow *photoautotrophically* with CO₂ and either H₂ or low levels of H₂S as reductant. Enrichment and isolation of purple nonsulfur bacteria is easy using a mineral salts medium supplemented with an organic acid as carbon source. Such media, inoculated with a mud, lake water, or sewage sample and incubated anaerobically in the light, invariably select for purple nonsulfur bacteria. Enrichment cultures can be made even more selective by omitting fixed nitrogen sources (for example, NH₄⁺) or organic nitrogen sources (for example, yeast extract or peptone) from the medium and supplying a gaseous headspace of N₂. Virtually all purple nonsulfur bacteria can fix N₂ and will thrive under such conditions, rapidly outcompeting other bacteria.

1.3 The Nitrifying Bacteria

Key Genera: *Nitrosomonas*, *Nitrobacter*

Many species of Bacteria are *chemolithotrophs*, organisms that can use inorganic electron donors as energy sources. Most *chemolithotrophs* are also

capable of *autotrophic* growth and in this way share a major physiological trait with *anoxygenic phototrophic* bacteria and *cyanobacteria*. We focus here on the best studied *chemolithotrophs*: those capable of oxidizing reduced nitrogen or sulfur compounds, ferrous iron (Fe^{2+}), or H_2 .

Ammonia and Nitrite Oxidizers

Bacteria able to grow *chemolithotrophically* at the expense of reduced inorganic nitrogen compounds are called *nitrifying bacteria* (Figure 7). Several genera are recognized on the basis of morphology and phylogeny as well as the particular steps in the oxidation sequences that they carry out (Table 3). Phylogenetically, the majority of nitrifying bacteria are scattered among four of the *Proteobacteria* classes: *Alpha*, *Beta*, *Gamma*, and *Delta*.

The genus *Nitrospira* forms its own *phylum* of Bacteria (Figure 1) and is related to other nitrifying bacteria in a metabolic sense only. Nevertheless, ecological studies of nitrification suggest that *Nitrospira* is the most abundant nitrifying bacterium in nature. In addition, certain *Archaea* oxidize ammonia (NH_3) as a *chemolithotrophic* substrate, and seem to be the dominant *nitrifiers* in the oceans, where ammonia levels are very low. No *chemolithotroph* is known that carries out the complete oxidation of NH_3 to nitrate (NO_3^-). Thus, nitrification results from the sequential activities of two

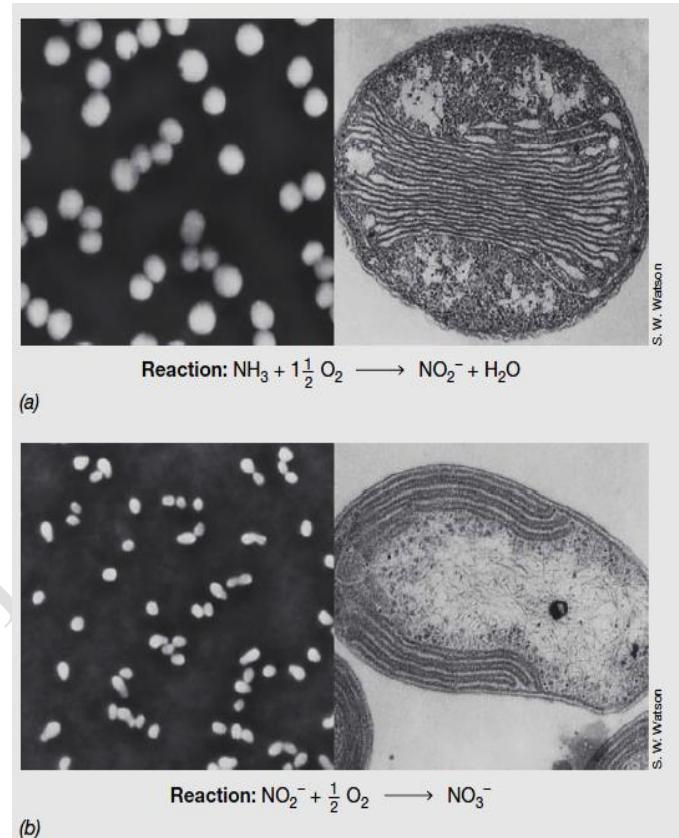


Figure 7: Nitrifying bacteria. (a) Phase photomicrograph (left) and electron micrograph (right) of the ammonia-oxidizing bacterium *Nitrosococcus oceanus*. A single cell is about 2 μm in diameter. (b) Phase-contrast photomicrograph (left) and electron micrograph (right) of the nitrite-oxidizing bacterium *Nitrobacter winogradskyi*. A cell is about 0.7 μm in diameter. Beneath each panel is shown the *chemolithotrophic* reaction that each organism catalyzes.

physiological groups of organisms, the ammonia-oxidizing bacteria (which oxidize NH₃ to nitrite, NO₂⁻) (Figure 7a), and the nitrite-oxidizing bacteria, the actual nitrate-producing bacteria, which oxidize NO₂⁻ to NO₃⁻ (Figure 7b). Ammonia-oxidizing bacteria typically have genus names beginning in *Nitroso-*, whereas genus names of nitrate producers begin with Nitro- (Table 3). Many species of nitrifying bacteria have internal membrane stacks (Figure 7) that closely resemble the photosynthetic membranes found in their close phylogenetic relatives, the purple phototrophic bacteria and the methane oxidizing (*methanotrophic*) bacteria. The membranes are the location of key enzymes in nitrification: *ammonia monooxygenase*, which oxidizes NH₃ to hydroxylamine (NH₂OH), and *nitrite oxidoreductase*, which oxidizes NO₂⁻ to NO₃⁻.

Table 1.3 Characteristics of the nitrifying bacteria

Characteristics	Genus	Phylogenetic group ^a	Primary habitats
Oxidize ammonia			
Gram-negative short to long rods, motile (polar flagella) or nonmotile; peripheral membrane systems	<i>Nitrosomonas</i>	Beta	Soil, sewage, freshwater, marine
Large cocci, motile; vesicular or peripheral membranes	<i>Nitrosococcus</i>	Gamma	Marine
Spirals, curved or lobed cells, motile (peritrichous flagella); no obvious membrane system	<i>Nitrosospira</i>	Beta	Soil, freshwater
Oxidize nitrite			
Short rods, reproduce by budding, occasionally motile (single subterminal flagellum); membrane system arranged as a polar cap	<i>Nitrobacter</i>	Alpha	Soil, freshwater, marine
Short rods forming cell aggregates; no internal membranes; psychrophile	<i>Nitrotoga</i>	Beta	Siberian permafrost
Long, slender rods, nonmotile; no internal membrane system	<i>Nitrospina</i>	Delta	Marine
Large cocci, motile (one or two subterminal flagella); membrane system randomly arranged in tubes	<i>Nitrococcus</i>	Gamma	Marine
Helical to vibrioid-shaped cells, nonmotile; no internal membranes	<i>Nitrospira</i>	<i>Nitrospira</i> group	Marine, sponges, soil, wastewater, hot springs

^aPhylogenetically, all nitrifying bacteria thus far examined are Proteobacteria, except for *Nitrospira*, which forms its own phylogenetic lineage (Figure 17.1 and Section 18.21), or certain marine crenarchaeotes (Section 19.11).

Ecology, Isolation, and Culture

The **nitrifying bacteria** are widespread in soil and water. They are present in highest numbers in habitats where NH₃ is abundant, such as sites with extensive protein decomposition (*ammonification*), and also in sewage treatment facilities. *Nitrifying bacteria* develop especially well in lakes and streams that receive inputs

of sewage or other wastewaters because these are frequently high in NH₃. Enrichment cultures of nitrifying bacteria can be achieved using mineral salts media containing NH₃ or NO₂⁻ as electron donors and bicarbonate (HCO₃⁻) as the sole carbon source. Because these organisms produce very little ATP from their electron donors, visible turbidity may not develop in cultures even after extensive nitrification has occurred. An easy means of monitoring growth is thus to assay for the production of NO₂⁻ (with NH₃ as electron donor) or NO₃⁻ (with NO₂⁻ as electron donor). Most of the *nitrifying bacteria* are *obligate chemolithotrophs* and *obligate aerobes*. Species of *Nitrobacter* are an exception and are able to grow *chemoorganotrophically* on acetate or pyruvate as the sole carbon and energy source. One group, the *anammox* bacteria, is phylogenetically distinct from the *nitrifiers* considered here and oxidizes NH₃ *anaerobically*.

1.4 Sulfur- and Iron-Oxidizing Bacteria

Key Genera: *Thiobacillus*, *Acidithiobacillus*, *Achromatium*, *Beggiatoa*

The ability to grow *chemolithotrophically* on reduced sulfur compounds is spread among organisms in four classes of *Proteobacteria* (**Table 4**). Two broad ecological classes of sulfur-oxidizing bacteria exist, those living at neutral pH and those living at acidic pH. Some of the *acidophiles* also have the ability to grow *chemolithotrophically* using ferrous iron (Fe²⁺) as an electron donor.

Thiobacillus and *Achromatium*

The genus *Thiobacillus* and related genera contain several gram negative, rod-shaped *Betaproteobacteria*, indistinguishable morphologically from most other gram-negative rods (**Figure 8a**); they are the best studied of the sulfur *chemolithotrophs*.

The electron donors most commonly used by the sulfur *chemolithotrophs* are H₂S, S⁰, and thiosulfate (S₂O₃²⁻). The oxidation of these substrates generates sulfuric acid (H₂SO₄), and thus several *thiobacilli* are acidophilic. One highly *acidophilic* species, *Acidithiobacillus ferrooxidans*, can also grow *chemolithotrophically* by the oxidation of Fe²⁺ and is a major biological agent for the oxidation of this metal. Iron pyrite (FeS₂) is a major natural source of Fe²⁺ as well as sulfide. The oxidation of FeS₂, especially in mining operations, can be both beneficial (because leaching of the ore releases the iron from the sulfide mineral) and ecologically disastrous (the environment can become acidic and contaminated with toxic metals such as aluminum, cadmium, and lead).

Achromatium is a spherical sulfur-oxidizing *chemolithotroph* that is common in freshwater sediments containing H₂S. Cells of *Achromatium* are large *cocci* that can have diameters of 10–100 μm (Figure 8b). Phylogenetic analyses of natural populations of *Achromatium* have shown that several species likely exist (probably each of distinct size), although pure cultures of this organism have not yet been achieved. *Achromatium* is a species of *Gammaproteobacteria* and is specifically related to purple sulfur bacteria, such as its *phototrophic* counterpart *Chromatium*. Like *Chromatium*, cells of *Achromatium* store S⁰ internally (Figure 8b); the granules later disappear as S⁰ is oxidized to SO₄²⁻. Cells of *Achromatium* also store

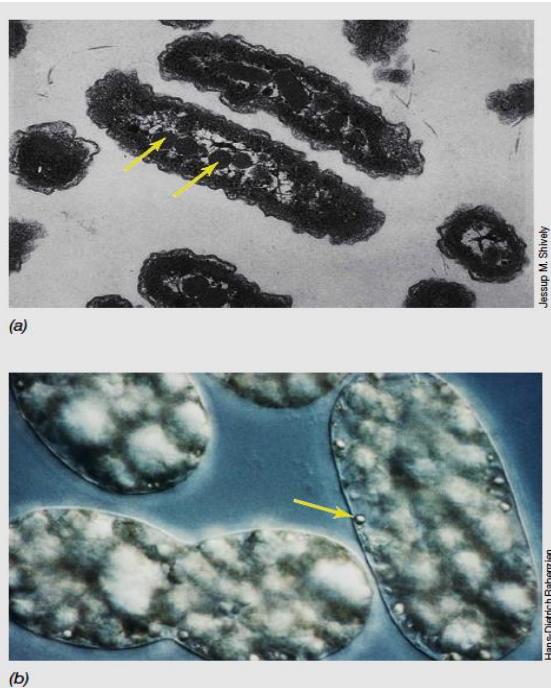


Figure 8: Nonfilamentous sulfur *chemolithotrophs*. (a) Transmission electron micrograph of cells of the *chemolithotrophic* sulfur oxidizer *Halothiobacillus neapolitanus*. A single cell is about 0.5 μm in diameter. Note the polyhedral bodies (carboxysomes) distributed throughout the cell (arrows). (b) *Achromatium*. Cells photographed by differential interference contrast microscopy. The small globular structures near the periphery of the cells (arrow) are elemental sulfur, and the large granules are calcium carbonate. A single *Achromatium* cell is about 25 μm in diameter.

large granules of calcite (CaCO_3) (Figure 8b), possibly as a carbon source (in the form of CO_2) for *autotrophic* growth.

Table 4 Physiological characteristics of sulfur-oxidizing chemolithotrophic bacteria

Genus and species	Inorganic electron donor	Range of pH for growth	Phylogenetic group ^a
Species growing poorly if at all in organic media			
<i>Thiobacillus thioparus</i>	H_2S , sulfides, S^0 , $\text{S}_2\text{O}_3^{2-}$	6–8	Beta
<i>Thiobacillus denitrificans</i> ^b	H_2S , S^0 , $\text{S}_2\text{O}_3^{2-}$	6–8	Beta
<i>Halothiobacillus neapolitanus</i>	S^0 , $\text{S}_2\text{O}_3^{2-}$	6–8	Gamma
<i>Acidithiobacillus thiooxidans</i>	S^0	2–4	Gamma
<i>Acidithiobacillus ferrooxidans</i>	S^0 , metal sulfides, Fe^{2+}	2–4	Gamma
Species growing well in organic media			
<i>Starkeya novella</i>	$\text{S}_2\text{O}_3^{2-}$	6–8	Alpha
<i>Thiomonas intermedia</i>	$\text{S}_2\text{O}_3^{2-}$	3–7	Beta
Filamentous sulfur chemolithotrophs			
<i>Beggiatoa</i>	H_2S , $\text{S}_2\text{O}_3^{2-}$	6–8	Gamma
<i>Thiotrix</i>	H_2S	6–8	Gamma
<i>Thioploca</i> ^c	H_2S , S^0	—	Gamma
Other genera			
<i>Achromatium</i>	H_2S	—	Gamma
<i>Thiomicrospira</i>	$\text{S}_2\text{O}_3^{2-}$, H_2S	6–8	Gamma
<i>Thiosphaera</i>	H_2S , $\text{S}_2\text{O}_3^{2-}$, H_2	6–8	Alpha
<i>Thermothrix</i>	H_2S , $\text{S}_2\text{O}_3^{2-}$, SO_3^{2-}	6.5–7.5	Beta
<i>Thiovulum</i> ^d	H_2S , S^0	6–8	Epsilon

^aAll are *Proteobacteria*.

^bFacultative aerobes; use NO_3^- as electron acceptor anaerobically.

^cPure cultures not yet available.

Culture

Some sulfur *chemolithotrophs* are obligate *chemolithotrophs*, locked into a lifestyle of using inorganic instead of organic compounds as electron donors. When growing in this fashion, they are also *autotrophs*, converting CO_2 into cell material by reactions of the Calvin cycle. **Carboxysomes** are often present in cells of obligate *chemolithotrophs* (Figure 8a). These structures contain high levels of Calvin cycle enzymes and probably increase the rate at which these organisms fix CO_2 . Other sulfur *chemolithotrophs* are *facultative chemolithotrophs*, *facultative* in the sense that they can grow either *chemolithotrophically* (and thus, also as *autotrophs*) or *chemoorganotrophically* (Table 4). Most species of *Beggiatoa*, however, can obtain energy from the oxidation of inorganic sulfur compounds but

lack enzymes of the Calvin cycle. They thus require organic compounds as carbon sources. Such a nutritional lifestyle is called ***mixotrophy***.

Beggiatoa

Organisms of this genus are filamentous, gliding, sulfur-oxidizing *Gammaproteobacteria*. Filaments of *Beggiatoa* are usually large in both diameter and length, consisting of many short cells attached end to end (**Figure 9**). Filaments then flex and twist so that many filaments may become intertwined to form a complex tuft. *Beggiatoa* is found in nature primarily in habitats rich in H₂S, such as sulfur springs (Figure 9b), decaying seaweed beds, mud layers of lakes, and waters polluted with sewage. In such environments, filaments of *Beggiatoa* are typically filled with S⁰ (Figure 9a). *Beggiatoa* are also common inhabitants of hydrothermal vents (underwater hot springs). Although a few strains of *Beggiatoa* are truly *chemolithotrophic autotrophs*, most grow best *mixotrophically* with reduced sulfur compounds as electron donors and organic compounds as carbon sources. An interesting habitat of *Beggiatoa* is the *rhizosphere* of plants (rice, cattails, and other swamp plants) living in flooded, and hence *anoxic*, soils. Such plants pump O₂ down into their roots, so a sharply defined *oxic–anoxic* boundary develops between the root and the soil. *Beggiatoa*



(a)



(b)

Figure 9: Filamentous sulfur-oxidizing bacteria. (a) Phase-contrast photomicrograph of a *Beggiatoa* species isolated from a sewage treatment plant. Note the abundant elemental sulfur granules in some of the cells. (b) Sulfur-oxidizing bacteria in the outflow of a small sulfide spring. The filamentous cells twist together to form thick streamers, and the white color is due to accumulated elemental sulfur.

and other sulfur bacteria develop at this interface and play a beneficial role for the plant by oxidizing (and thus detoxifying) the H₂S.

Thioploca* and *Thiothrix

Other filamentous sulfur-oxidizing *Gammaproteobacteria* include *Thioploca* (Figure 10) and *Thiothrix* (Figure 11). *Thioploca* is a large, filamentous sulfur-oxidizing chemolithotroph that forms cell bundles surrounded by a common sheath (Figure 10). Thick mats of a marine *Thioploca* species have been found on the coastlines of the Americas and Australia. Ecological studies of these organisms show that they can use the anoxic oxidation of H₂S coupled to the reduction of nitrate (NH₄⁺). Cells of *Thioploca* can accumulate humic acids and this NO₃⁻ can then support extended periods of growth using humic acids as electron donor. It is thought that these *Thioploca* strains produce CO₂ and also play a major role in sulfur cycling in the environment. *Thiothrix* is a filamentous sulfur-oxidizing bacterium that forms filaments group together at their ends by arrangements called rosettes (Figure 11b). *Thiothrix* is an *obligately aerobic mixotroph*, and in this aspect it is similar to *Beggiatoa*.

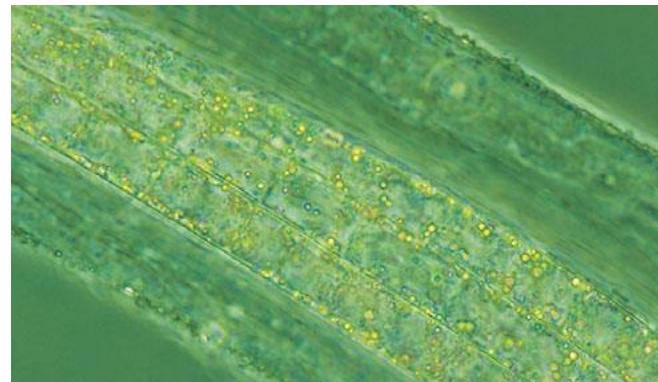
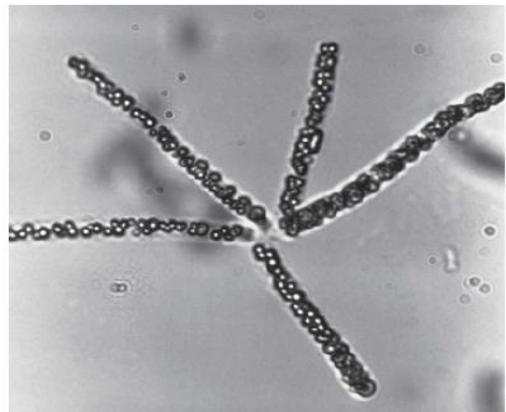


Figure 10: Cells of a large marine *Thioploca* species. Cells contain sulfur granules (yellow) and are about 40–50 μm wide.



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Figure 11: *Thiothrix*. (a) A sulfide-containing artesian spring in Florida (USA). The outside of the spring is coated with a mat of *Thiothrix*. The mat is about 1.5 m in diameter. (b) Phase-contrast photomicrograph of a rosette of cells of *Thiothrix* isolated from the spring. Note the internal sulfur globules produced from the oxidation of sulfide. Each filament is about 4 μm in diameter.

1.5 Hydrogen-Oxidizing Bacteria

Key Genera: *Ralstonia, Paracoccus*

Many bacteria can grow with H₂ as sole electron donor and O₂ as electron acceptor in their energy metabolism: $H_2 + \frac{1}{2} O_2 \rightarrow H_2O$ $\Delta G^0 = -237 \text{ kJ}$

Most of these organisms, known collectively as the “**hydrogen bacteria**,” can also grow *autotrophically* (using reactions of the Calvin cycle to incorporate CO₂) and are grouped together here as the *chemolithotrophic* hydrogen-oxidizing bacteria. All hydrogen bacteria contain one or more **hydrogenase** enzymes that function to bind H₂ and use it either to produce ATP or for reducing power for *autotrophic* growth (**Table 5**).

Table 5 Differential characteristics of a few common species of hydrogen-oxidizing bacteria

Genus and species	Denitrification	Growth on fructose	Motility	Phylogenetic group ^a	Other characteristics
Gram-negative					
<i>Acidovorax facilis</i>	—	+	+	Beta	Membrane-bound hydrogenase
<i>Ralstonia eutropha</i>	+	+	+	Beta	Membrane-bound and cytoplasmic hydrogenases
<i>Achromobacter xylosoxidans</i>	—	+	+	Beta	Membrane-bound and cytoplasmic hydrogenases
<i>Aquaspirillum autotrophicum</i>	—	—	+	Beta	Membrane-bound hydrogenase
<i>Pseudomonas carboxydovorans</i>	—	—	+	Gamma	Membrane-bound hydrogenase; also oxidizes CO
<i>Hydrogenophaga flava</i>	—	+	+	Beta	Colonies are bright yellow
<i>Paracoccus denitrificans</i>	+	+	—	Alpha	Membrane-bound hydrogenase; strong denitrifier
<i>Aquifex pyrophilus</i>	+	—	+	Aquifex group ^b	Hyperthermophile, grows microaerophilically or anaerobically (with NO ₃ [—]), obligate chemolithotroph; also uses S ⁰ or S ₂ O ₃ ^{2—} as electron donor
<i>Hydrogenobacter thermophilus</i>	—	—	—	Aquifex group ^b	As for <i>Aquifex</i> , but obligate aerobe (microaerophile)
Gram-positive					
<i>Bacillus schlegelii</i>	—	—	+	Firmicutes ^c	Produces endospores; thermophile; also uses CO or S ₂ O ₃ ^{2—} as electron donor
<i>Arthrobacter</i> sp.	—	+	—	Actinobacteria ^d	Membrane-bound hydrogenase
<i>Mycobacterium gordoneae</i>	—	?	—	Actinobacteria ^e	Acid-fast; colonies yellow to orange

^aAerobic hydrogen bacteria are *Proteobacteria* except as indicated.

Different hydrogen-oxidizing *Proteobacteria* are scattered among the *Alpha*, *Beta*, and *Gamma* subclasses. These organisms should be distinguished from the many strictly *anaerobic prokaryotes* that oxidize H₂ in *anaerobic* respirations; for example, *acetogens*, *methanogens*, and sulfate-reducing bacteria. Both gram-

positive and gram-negative hydrogen bacteria are known, with the best-studied representatives classified in the genera *Ralstonia* (Figure 12), *Pseudomonas*, and *Paracoccus* (Table 5). *Paracoccus denitrificans* can also oxidize H₂ anaerobically with nitrate (NO₃) as electron acceptor, forming N₂ (*denitrification*), and has been particularly well studied for its bioenergetics of electron transport and generation of a proton motive force.

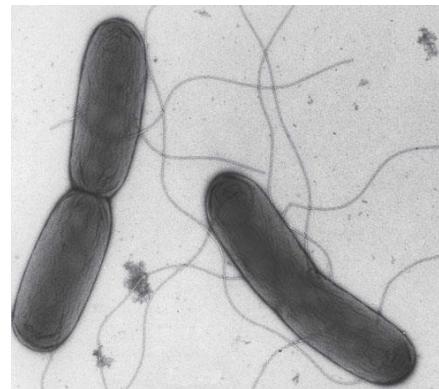


Figure 12: Hydrogen bacteria. Transmission electron micrograph of negatively stained cells of the hydrogen-oxidizing *chemolithotroph Ralstonia eutropha*. A cell is about 0.6 μm in diameter and contains several flagella.

Physiology and Ecology of Hydrogen Bacteria

When growing *chemolithotrophically* on H₂, most hydrogen bacteria grow best under *microaerophilic* (5–10% O₂) conditions because *hydrogenases* are typically oxygen-sensitive. The element nickel (Ni²⁺) must be present in the medium for *chemolithotrophic* growth of hydrogen bacteria because virtually all *hydrogenases* contain Ni²⁺ as a key metal cofactor. A few hydrogen bacteria also fix nitrogen, making possible their culture in a mineral salts medium supplied with only H₂, O₂, CO₂, and N₂ as carbon, energy, and nitrogen sources!

Virtually all hydrogen bacteria are *facultative chemolithotrophs*, meaning that they can also grow *chemoorganotrophically* with organic compounds as energy sources. Hydrogen-oxidizing bacteria can be enriched if a small amount of mineral salts medium containing trace metals (especially Ni²⁺ and Fe²⁺) is inoculated with soil or water and incubated in a large, sealed flask containing a headspace of 5% O₂, 10% CO₂, and 85% H₂. When the liquid becomes turbid, plates of the same medium are streaked and incubated in a glass jar containing the same gas mixture (one must exercise care here, as mixtures of O₂ and H₂ are potentially explosive).

CO Oxidation

Some hydrogen bacteria can grow *aerobically* on carbon monoxide (CO) as electron donor. CO-oxidizing bacteria, called ***carboxydrophic*** bacteria, grow *autotrophically* using the Calvin cycle to fix CO₂ generated from the oxidation of CO. Electrons from the oxidation of CO to CO₂ by the enzyme carbon monoxide dehydrogenase travel through an electron transport chain that forms a proton motive force. Interestingly, CO is a potent inhibitor of many cytochromes, acting as a respiratory poison. However, *carboxydrophic* bacteria get around this problem by synthesizing CO-resistant cytochromes and are thus immune to any toxic effects of CO. Like the hydrogen bacteria, virtually all *carboxydrophic* bacteria also grow *chemoorganotrophically* by oxidizing organic compounds, a likely indication that CO levels are quite variable in nature and a backup means of energy metabolism is essential.

CO consumption by *carboxydrophic* bacteria on a global basis is a significant ecological process. Although much CO is generated from human and other sources, CO levels in air have not risen significantly over many years. Because the most significant releases of CO (primarily from automobile exhaust, incomplete combustion of fossil fuels, and the catabolism of lignin, a plant product) are in oxic environments, *carboxydrophic* bacteria in the upper layers of soil probably represent the most significant sink for CO in nature.

1.6 Methanotrophs and Methylotrophs

Key Genera: *Methylomonas*, *Methylobacter*

Methane (CH_4) is found extensively in nature. It is produced in *anoxic* environments by *methanogenic Archaea* and is a major gas of *anoxic* muds, marshes, *anoxic* zones of lakes, the rumen, and the mammalian intestinal tract. Methane is the major constituent of “natural gas” widely used as a heating and industrial fuel, and is also present in many coal formations.

Methanotrophs oxidize methane and a few other one-carbon compounds as electron donors in energy metabolism and as carbon sources. *Methanotrophs* grow **aerobically** and are widespread in soils and waters. They exhibit diverse morphologies but are related in terms of their phylogeny and ecology. In marine sediments and a few other *anoxic* environments, methane is oxidized under strictly *anoxic* conditions by a consortium of methanogenic *Archaea* and sulfate-reducing bacteria, but we consider here only the **aerobic methanotrophs**.

C₁ Metabolism

A list of compounds catabolized by *methanotrophs* is given in **Table 6**. From a biochemical point of view, these compounds share a key characteristic, the absence of carbon–carbon bonds. Thus, in *methanotrophs* all organic compounds in the cell must be synthesized from C₁ precursors. Organisms that can grow using carbon compounds that lack C—C bonds are called **methylotrophs**. Many but not all *methylotrophs* are also *methanotrophs*. However, *methanotrophs* are unique in that they can grow not only on some of the more oxidized one-carbon compounds, but also on methane.

Methanotrophs possess a key enzyme, **methane monooxygenase**, which catalyzes the incorporation of an atom of oxygen from O₂ into CH₄, forming methanol (CH₃OH). The requirement for O₂ as a reactant in the initial oxygenation of CH₄ thus explains why these *methanotrophs* are **obligate aerobes**. Most

methanotrophs are obligate C₁ utilizers, unable to use compounds containing carbon–carbon bonds. By contrast, most *nonmethanotrophic methylotrophs* can use organic acids, ethanol, and sugars. **Methane-oxidizing** bacteria are virtually unique among bacteria in possessing relatively large amounts of **sterols**. Sterols are rigid planar molecules found in the cytoplasmic and other membranes of *eukaryotes* but are absent from most bacteria. Sterols may be an essential part of the complex internal membrane system for methane oxidation. The only other group of bacteria in which sterols are widely distributed is in the cell wall-less *mycoplasmas*.

Classification of Methanotrophs

Table 6 gives a taxonomic overview of the *methanotrophs*. These bacteria were initially distinguished on the basis of morphology and formation of resting stages. However, now they are classified into two major groups based on their internal cell structure, phylogeny, and carbon assimilation pathway. Type I *methanotrophs* assimilate one-carbon compounds via the ribulose monophosphate cycle and are phylogenetically *Gammaproteobacteria*. By contrast, type II *methanotrophs* assimilate C₁ intermediates via the serine pathway and are phylogenetically *Alphaproteobacteria* (Table 6).

Table 6 Some characteristics of methanotrophic bacteria

Organism	Morphology	Phylogenetic group ^a	Internal membranes ^b	Carbon assimilation pathway ^c	N ₂ fixation
<i>Methylomonas</i>	Rod	Gamma	I	Ribulose monophosphate	No
<i>Methylomicrobium</i>	Rod	Gamma	I	Ribulose monophosphate	No
<i>Methylobacter</i>	Coccus to ellipsoid	Gamma	I	Ribulose monophosphate	No
<i>Methylococcus</i>	Coccus	Gamma	I	Ribulose monophosphate and Calvin cycle	Yes
<i>Methylosinus</i>	Rod or vibrioid	Alpha	II	Serine	Yes
<i>Methylocystis</i>	Rod	Alpha	II	Serine	Yes
<i>Methylocella^d</i>	Rod	Alpha	II	Serine	Yes
<i>Methylacidiphilum^d</i>	Rod	Verrucomicrobiaceae ^d (see Figure 17.1)	Membrane vesicles	Serine and Calvin cycle	Yes

^aAll except for *Methylacidiphilum* are *Proteobacteria*.

^bInternal membranes: type I, bundles of disc-shaped vesicles distributed throughout the organism; type II, paired membranes running along the periphery of the cell.

Both groups of *methanotrophs* contain extensive internal membrane systems for methane oxidation. Membranes in type I *methanotrophs* are arranged as bundles of disc-shaped vesicles distributed throughout the cell (**Figure 13b**). Type II species possess paired membranes running along the periphery of the cell (Figure 13a). The key enzyme methane *monooxygenase* is located in these membranes. The genus *Methylacidiphilum* contains a phylogenetically and physiologically unique *methanotroph*. Species in this genus are *thermophilic* and *extremely acidophilic*, growing optimally at pH 2 and capable of growth below pH 1. *Methylacidiphilum* inhabits acidic geothermal environments where CH₄ is released in vented gas; at the interface of the *anoxic* geothermal gas and the atmosphere, cells have the two key substrates they need (CH₄ plus O₂) to grow as *methanotrophs*. *Methylacidiphilum* is related to species of the genus *Verrucomicrobium*, an organism that forms its own lineage of Bacteria, and grows in laboratory culture only on CH₄ or CH₃OH at acidic pH. *Methylacidiphilum* also fixes nitrogen, as do many other *methanotrophs* (Table 6), but at the acidic pH values at which *Methylacidiphilum* thrives, it is probably one of the most *acidophilic* of all known *nitrogen-fixing* bacteria.

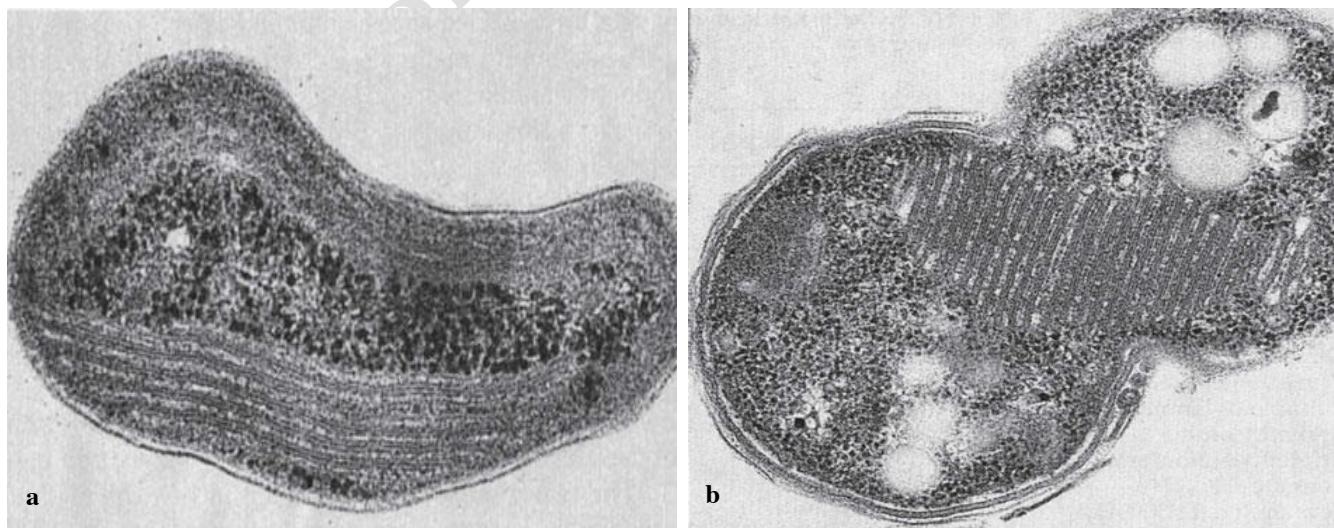


Figure 13: Methanotrophs. (a) Electron micrograph of a cell of *Methylosinus*, illustrating a type II membrane system. Cells are about 0.6 μm in diameter. (b) Electron micrograph of a cell of *Methylococcus capsulatus*, illustrating a type I membrane system. Cells are about 1 μm in diameter.

Ecology and Isolation

Methanotrophs are widespread in aquatic and terrestrial environments, being found wherever stable sources of CH₄ are present. Methane produced in the *anoxic* regions of lakes rises through the water column, and *methanotrophs* are often concentrated in a narrow band at the zone where CH₄ and O₂ meet. Methane-oxidizing bacteria therefore play an important role in the carbon cycle, converting CH₄ derived from *anoxic* decomposition back into cell material and CO₂. *Methanotrophic* bacteria and certain marine mussels and sponges have developed symbiotic relationships. Some marine mussels live in the vicinity of hydrocarbon seeps on the seafloor, places where CH₄ is released in substantial amounts. Isolated mussel gill tissues consume CH₄ at high rates in the presence of O₂. In these tissues, *coccoid*-shaped bacteria are present in high numbers (**Figure 14**). The bacterial symbionts contain intracytoplasmic membranes typical of *methanotrophs* (Figure 14b). The symbionts reside in vacuoles within animal cells near the gill surface, which probably ensures an effective gas exchange with seawater, and the fact that these symbionts are indeed *methanotrophs* has been shown by phylogenetic analyses. Assimilated CH₄ is distributed throughout the animal by the excretion of organic compounds by the *methanotrophs*. These *methanotrophic* symbioses are therefore conceptually quite similar to those that develop between *sulfide-oxidizing chemolithotrophs* and hydrothermal vent tube worms and giant clams.

All that is needed to enrich *methanotrophs* is a mineral salts medium containing a headspace about 50% each of CH₄ and air. Once good growth is obtained, purification can be achieved by repeated streaking on mineral salts agar plates incubated in a CH₄–air mixture. Colonies appearing on the plates are typically of two types: *nonmethanotrophic chemoorganotrophs* growing on traces of organic matter in the medium, which appear in 1–2 days, and *methanotrophs*,

which appear after about a week. The colonies of some *methanotrophs* are pink from the presence of various carotenoid pigments and high levels of cytochromes in their membranes, and this feature can assist in identifying these organisms on plates.

Methanotrophs and Ammonia-Oxidizing Bacteria

Besides CH₄, *methanotrophs* can also oxidize ammonia (NH₃), and ammonia-oxidizing bacteria can also oxidize CH₄; however, neither group can actually grow using the other group's substrate as electron donor. It has been hypothesized that *methanotrophic* bacteria evolved from *ammonia-oxidizing* bacteria via selection for the conversion of an *ammonia monooxygenase* into a *methane monooxygenase*. The fact that *methanotrophs* and *nitrifiers* have similar internal membrane systems and are phylogenetically closely related supports this theory. However, it has also been found that *methanotrophic bacteria* contain some of the same genes and make some of the same proteins as methanogenic (methane-producing) *Archaea*, and so the evolution of *methanotrophy* is still very much an open question.

III- Aerobic and Facultatively Aerobic Chemoorganotrophic *Proteobacteria*

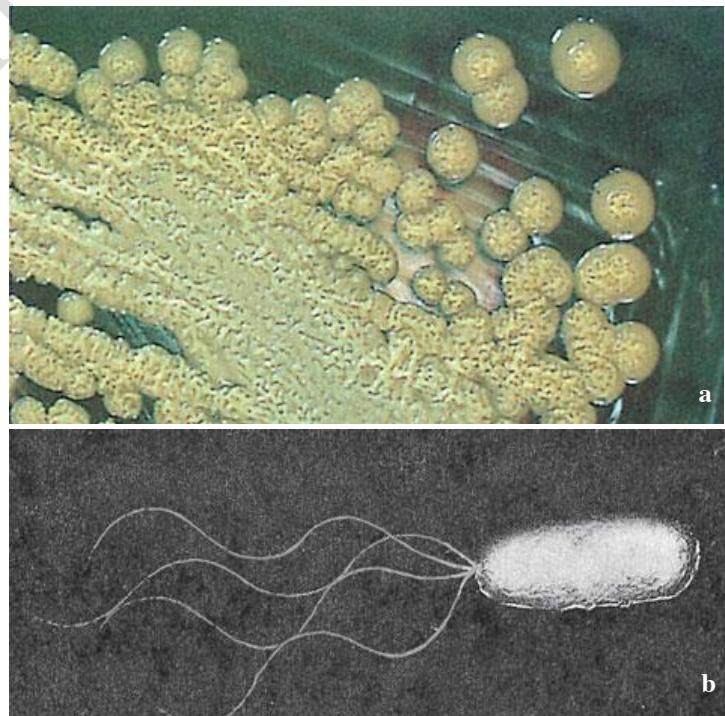


Figure 14: Typical pseudomonad colonies and cell morphology of pseudomonads. (a) Photograph of colonies of *Burkholderia cepacia* on an agar plate. (b) Shadow-cast transmission electron micrograph of a *Pseudomonas* cell. The cell measures about 1 μm in diameter.

The next few groups to be considered are the classic examples of *chemoorganotrophic* bacteria that carry out respiratory metabolisms. Here we will meet the pseudomonads, the enteric bacteria, the aerobic nitrogen-fixing bacteria, and many of their close relatives.

1.7 *Pseudomonas* and the Pseudomonads

Key Genera: *Pseudomonas*, *Burkholderia*, *Zymomonas*, *Xanthomonas*

All the genera in this group are straight or slightly curved gram negative, *chemoorganotrophic* rods with polar flagella (Figure 14). Common genera are *Pseudomonas*, *Comamonas*, *Ralstonia*, and *Burkholderia*, discussed in some detail here. Other important genera include *Xanthomonas*, *Zoogloea*, and *Gluconobacter*. Phylogenetically, the pseudomonads scatter within the *Proteobacteria* (Table 7).

Table 7 Subgroups and characteristics of pseudomonads

Group	Phylogenetic group ^a	Characteristics
Fluorescent subgroup	Gamma	Most produce water-soluble, yellow-green fluorescent pigments; do not form poly-β-hydroxybutyrate; single DNA homology group <i>Pseudomonas aeruginosa</i> <i>Pseudomonas fluorescens</i> <i>Pseudomonas putida</i> <i>Pseudomonas syringae</i> <i>Pseudomonas stutzeri</i>
Acidovorans subgroup	Beta	Nonpigmented; form poly-β-hydroxybutyrate; tuft of polar flagella; do not use carbohydrates; single DNA homology group <i>Deltiella acidovorans</i> <i>Comamonas testosteroni</i>
Pseudomallei-cepacia subgroup	Beta	No fluorescent pigments; tuft of polar flagella; forms poly-β-hydroxybutyrate; single DNA homology group <i>Burkholderia cepacia</i> <i>Burkholderia pseudomallei</i> <i>Burkholderia mallei</i>
Diminuta-vesicularis subgroup	Alpha	Single flagellum of very short wavelength; require vitamins (pantothenate, biotin, B₁₂) <i>Brevundimonas diminuta</i> <i>Brevundimonas vesicularis</i>
Ralstonia subgroup	Beta	Plant pathogen <i>Ralstonia solanacearum</i> <i>Pelomonas saccharophila</i>
<i>Stenotrophomonas maltophilia</i>	Gamma	Requires methionine; does not use NO ₃ ⁻ as N source; oxidase-negative
Others		Not pathogenic; unusual ecology or physiology
<i>Zoogloea</i>	Beta	Forms an extracellular fibrillar polymer that causes the cells to aggregate into distinctive flocs as a major component of activated sewage sludge (Section 35.2).
<i>Gluconobacter</i>	Alpha	Incompletely oxidizes glucose to gluconic acid or ethanol to acetic acid; important in vinegar production
<i>Zymomonas</i>	Alpha	Obligately fermentative; ferments glucose to ethanol plus CO ₂

^aAll pseudomonads are species of *Proteobacteria*.

Characteristics of Pseudomonads

Some major distinguishing characteristics of the pseudomonad group include an obligately respiratory metabolism, the absence of gas formation from glucose, and a positive oxidase test, all of which help to distinguish pseudomonads from enteric bacteria. Although obligately respiratory, many pseudomonads can still grow under *anoxic* conditions with nitrate, fumarate, or many of the other electron acceptors that support anaerobic respiration. Key species of the genus *Pseudomonas* and related genera are defined on the basis of phylogeny and various physiological and other phenotypic characteristics, as outlined in Table 7. Pseudomonads typically have very simple nutritional requirements and one of their characteristic properties is the ability to use many different organic compounds as carbon and energy sources; some species utilize over 100 different compounds. On the other hand, pseudomonads generally lack the hydrolytic enzymes necessary to break down polymers into their component monomers.

The genomes of these nutritionally versatile pseudomonads encode numerous inducible operons, some of which encode a large number of enzymes to handle the catabolism of the many different organic substrates that are used. The pseudomonads are ecologically important in soil and water and are probably responsible for the degradation of many low-molecular-weight compounds derived from the breakdown of plant and animal materials in *oxic* habitats. They are also capable of catabolizing many xenobiotic (not naturally occurring) compounds, such as pesticides and other toxic chemicals, and are thus important agents of bioremediation in the environment.

Pathogenic Pseudomonads

A number of pseudomonads are pathogenic (Table 8). Among the fluorescent pseudomonads, the species *Pseudomonas aeruginosa* is frequently associated with infections of the urinary and respiratory tracts in humans. *P.*

aeruginosa infections are also common in patients receiving treatment for severe burns or other traumatic skin damage and in people suffering from cystic fibrosis.

Table 8 Pathogenic pseudomonads

Species	Relationship to disease
Animal pathogens	
<i>Pseudomonas aeruginosa</i>	Opportunistic pathogen, especially in hospitals; in patients with metabolic, hematologic, and malignant diseases; hospital-acquired (nosocomial) infections from catheterizations, tracheostomies, lumbar punctures, and intravenous infusions; in patients given prolonged treatment with immunosuppressive agents, corticosteroids, antibiotics, and radiation; may contaminate surgical wounds, abscesses, burns, ear infections, lungs of patients treated with antibiotics; lungs of those with cystic fibrosis; primarily a soil organism
<i>Pseudomonas fluorescens</i>	Rarely pathogenic, as it does not grow well at 37°C; may grow in and contaminate blood and blood products under refrigeration
<i>Stenotrophomonas maltophilia</i>	A ubiquitous, free-living organism that is a common nosocomial pathogen
<i>Burkholderia cepacia</i>	Isolated from humans and from environmental sources of medical importance; also plant pathogen, causes onion bulb rot
<i>Burkholderia pseudomallei</i>	Causes melioidosis, a disease endemic in animals and humans in Southeast Asia
<i>Burkholderia mallei</i>	Causes glanders, a disease of horses that is occasionally transmitted to humans
<i>Pseudomonas stutzeri</i>	Often isolated from humans and environmental sources; may live saprophytically in the body
Plant pathogens	
<i>Ralstonia solanacearum</i>	Causes wilts of many cultivated plants (for example, potato, tomato, tobacco, peanut)
<i>Pseudomonas syringae</i>	Attacks foliage, causing chlorosis and necrotic lesions on leaves; rarely found free in soil
<i>Pseudomonas marginalis</i>	Causes soft rot of various plants; active pectinolytic species
<i>Xanthomonas campestris</i>	Causes necrotic lesions on foliage, stems, fruits; also causes wilts and tissue rots; rarely found free in soil

P. aeruginosa is naturally resistant to many of the widely used antibiotics, so treatment of infections is often difficult. Resistance is due to a resistance transfer plasmid (R plasmid), which is a plasmid carrying genes encoding proteins that detoxify various antibiotics or pump them out of the cell. *P. aeruginosa* is commonly found in the hospital environment and can easily infect patients receiving treatment for other illnesses (healthcare-associated infections). Polymyxin, an antibiotic not ordinarily used in human therapy because of its toxicity, is effective against *P. aeruginosa* and is used in certain medical situations. Certain species of *Pseudomonas*, *Ralstonia*, and *Burkholderia* and the genus *Xanthomonas* are well-known plant pathogens (phytopathogens) (Table 8). Phytopathogens frequently inhabit nonhost plants (in which disease symptoms are not apparent) and from there are transmitted to host plants and initiate infection. Disease symptoms vary considerably, depending on the particular phytopathogen and host plant. The pathogen releases plant toxins, lytic enzymes, plant growth factors, and other substances that destroy or distort plant tissue. In many cases the

disease symptoms help identify the phytopathogen. Thus, *Pseudomonas syringae* is typically isolated from leaves showing chlorotic (yellowing) lesions, whereas *Pseudomonas marginalis*, a typical “soft-rot” pathogen, infects stems and shoots, but rarely leaves.

Zymomonas

The genus *Zymomonas* consists of large, gram-negative rods that carry out a vigorous fermentation of sugars to ethanol. Although distinct from other pseudomonads by its strictly fermentative metabolism, *Zymomonas* shows phylogenetic affiliation with these organisms (Table 7), and like other pseudomonads it employs the Entner–Doudoroff pathway for glucose catabolism. *Zymomonas* carries out alcoholic fermentations of various plant saps, and in many tropical areas of South and Central America, Africa, and Asia it is found in various fermented beverages made from plant saps, such as pulque (agave), palm sap, sugarcane juice, and honey. Although *Zymomonas* is rarely the sole organism responsible for these alcoholic fermentations, it is probably responsible for the production of most of the ethanol in these beverages where yeasts are present in only low numbers. *Zymomonas* is also responsible for spoilage of fruit juices, such as apple and pear ciders, and is also a frequent constituent of the bacterial flora of spoiled beer.

1.8 Acetic Acid Bacteria

Key Genera: *Acetobacter*, *Gluconobacter*

The acetic acid bacteria comprise gram-negative, obligately *aerobic*, motile rods that carry out the incomplete oxidation of alcohols and sugars, leading to the accumulation of organic acids as end products. With ethanol (C_2H_2OH) as a substrate, acetic acid ($C_2H_4O_2$) is produced, which gives the acetic acid bacteria their name. As one would expect, acetic acid bacteria are tolerant of acidic

conditions; most strains can grow well at pH values lower than 5. The acetic acid bacteria are a heterogeneous assemblage of *Alphaproteobacteria*, comprising both *peritrichously* flagellated (*Acetobacter*) and *polarly* flagellated (*Gluconobacter*) organisms. In addition to flagellation, *Acetobacter* differs physiologically from *Gluconobacter* in being able to further oxidize the acetic acid it forms to CO₂; that is, with *Gluconobacter*, acetic acid is a “deadend” product.

Ecology and Industrial Uses

The acetic acid bacteria are commonly found in fermenting fruit juices, such as hard cider or wine, or in beer. Colonies of acetic acid bacteria can be recognized on calcium carbonate (CaCO₃) agar plates containing ethanol, because the acetic acid produced dissolves and causes a clearing of the otherwise insoluble CaCO₃ (Figure 15). Cultures of acetic acid bacteria are used in the commercial production of vinegar. In addition to ethanol, the acetic acid bacteria carry out an incomplete oxidation of some higher alcohols and sugars. For instance, glucose is oxidized to gluconic acid, galactose to galactonic acid, arabinose to arabonic acid, and so on. This property of “*underoxidation*” is exploited in the industrial manufacture of ascorbic acid (vitamin C). Ascorbic acid can be formed from sorbose, but sorbose is difficult to synthesize chemically. It is, however, conveniently obtainable from acetic acid bacteria, which oxidize sorbitol (a readily available sugar alcohol) to sorbose. Another interesting property of some acetic acid bacteria is their ability to synthesize cellulose. The cellulose formed does not differ significantly from plant cellulose, with the exception that it is pure

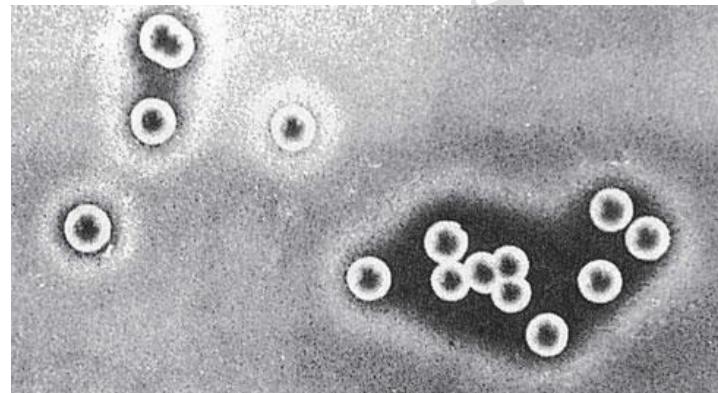


Figure 15: Colonies of *Acetobacter aceti* on calcium carbonate (CaCO₃) agar containing ethanol as electron donor. Note the clearing around the colonies due to the dissolution of CaCO₃ by the acetic acid produced.

and not mixed in with other polymers like the hemicelluloses, pectin, or lignins of plants. Cellulose from acetic acid bacteria is formed as a matrix outside the cell wall and causes cells to become embedded in a tangled mass of cellulose microfibrils. When these species of acetic acid bacteria grow in an unshaken vessel, they form a surface pellicle of cellulose in which the bacteria develop. Because these bacteria are *obligate aerobes*, the ability to form such a pellicle may be a means by which the organisms remain at the surface of the liquid where oxygen is readily available.

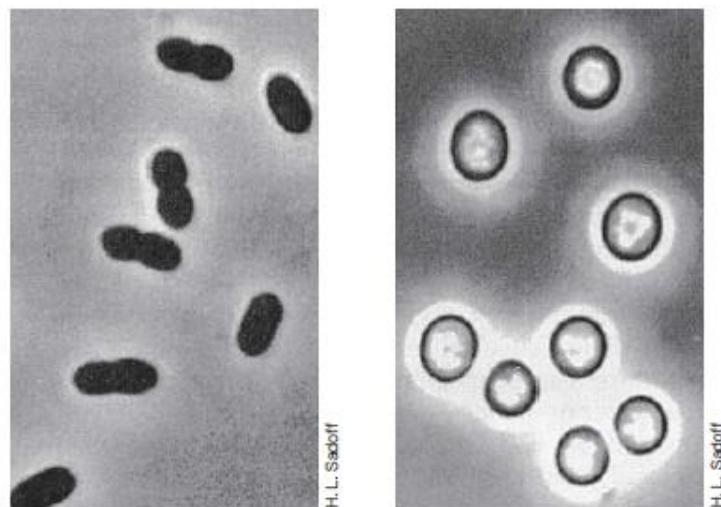
Table 9 Genera of free-living, aerobic nitrogen-fixing bacteria

Characteristics	Genus
Gammaproteobacteria	
Large rod; produces cysts; primarily found in neutral to alkaline soils	<i>Azotobacter</i>
Large rod; no cysts; primarily aquatic	<i>Azomonas</i>
Alphaproteobacteria	
Microaerophilic rod; associates with plants	<i>Azospirillum</i>
Pear-shaped rod with large lipid bodies at each end; produces extensive slime; inhabits acidic soils	<i>Beijerinckia</i>
Betaproteobacteria	
Small curved cells; no cysts	<i>Azoarcus</i>
Very thin curved cells; no cysts	<i>Azovibrio</i>
Very thin rods to vibrios; no cysts	<i>Azospira</i>
Cells form coils up to 50 µm long; no cysts	<i>Azonexus</i>
Rods; form coarse, wrinkled colonies	<i>Dexia</i>

1.9 Free-Living Aerobic Nitrogen-fixing Bacteria

Key Genera: *Azotobacter*, *Azomonas*, *Beijerinckia*

A variety of free-living *chemoorganotrophic* bacteria inhabit soil and are capable of aerobic nitrogen (N_2) fixation. The genus *Azotobacter* (Figure 16) was the first such organism and was discovered by the Dutch microbiologist Martinus Beijerinck, early in the twentieth century. Beijerinck employed an aerobic



(a) (b)

Figure 16: *Azotobacter vinelandii*. (a) Vegetative cells and (b) cysts visualized by phase-contrast microscopy. A cell measures about 2 µm in diameter and a cyst about 3 µm.

enrichment culture devoid of a combined nitrogen source but exposed to air. Phylogenetically, free-living nitrogen-fixing bacteria are *Alpha*-, *Beta*-, or *Gammaproteobacteria* (**Table 9**).

Taxonomy

The major free-living nitrogen-fixing bacteria that have been well studied include *Azotobacter*, *Azospirillum*, and *Beijerinckia*. *Azotobacter* cells are large rods or cocci, many isolates being almost the size of yeasts (eukaryotes), with diameters of 2–4 μm or more, and some species are motile by peritrichous flagella. When they are growing on N_2 as a nitrogen source, extensive capsules or slime layers are typically produced by species of free living nitrogen-fixing bacteria (**Figure 17**). This layer helps protect the enzyme *nitrogenase* in the cytoplasm. Despite the fact that *Azotobacter* is an *obligate aerobe*, its *nitrogenase*, the enzyme that catalyzes N_2 fixation, is O_2 -sensitive. It is thought that the high respiratory rate characteristic of *Azotobacter* cells and the abundant capsular slime they produce help protect *nitrogenase* from O_2 . *Azotobacter* is able to grow on many different carbohydrates, alcohols, and organic acids, and metabolism is strictly oxidative. All species fix nitrogen, but can also grow on simple forms of combined nitrogen. *Azotobacter* can form resting structures called *cysts* (Figure 16b). Like bacterial endospores, *Azotobacter cysts* show negligible endogenous respiration and are resistant to desiccation, mechanical disintegration, and ultraviolet and ionizing

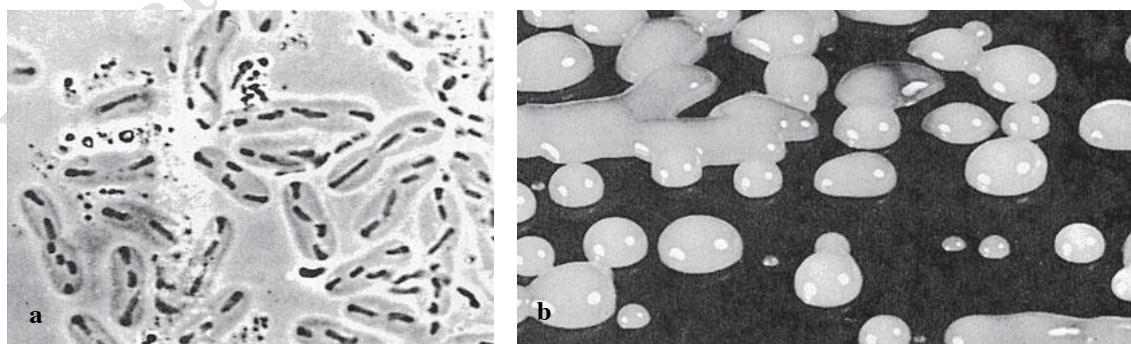


Figure 17: Examples of slime production by free-living N_2 -fixing bacteria. (a) Cells of *Derrxia gummosa* encased in slime. Cells are about 1–1.2 μm wide. (b) Colonies of *Beijerinckia* species growing on a carbohydrate-containing medium. Note the raised, glistening appearance of the colonies due to abundant capsular slime.

radiation. In contrast to endospores, however, cysts are not very heat resistant, and they are not completely dormant because they rapidly oxidize carbon sources if supplied. The remaining major genera of free-living nitrogen fixers include *Azomonas*, a genus of large *coccus* to *rod*-shaped bacteria that resemble *Azotobacter*, except that they do not produce *cysts* and are primarily aquatic, and *Beijerinckia* and *Dexia* (**Figure 18**), two genera that grow well in acidic soils. *Azospirillum*, a *rod*- to *spirillum*-shaped nitrogen-fixing bacterium that forms nonspecific symbiotic associations with plants, in particular, corn (Table 9), rounds out this group.

***Azotobacter* and Alternative Nitrogenases**

We considered the important process of biological N₂ fixation in, and learned of the central importance of the metals molybdenum (Mo) and iron (Fe) to the enzyme nitrogenase. The species *Azotobacter chroococcum* was the first nitrogen-fixing bacterium shown capable of growth on N₂ in the absence of molybdenum. It was shown in *A. chroococcum* that either of two “alternative nitrogenases” are formed when Mo limitation prevents the normal Mo nitrogenase from being synthesized. These nitrogenases are less efficient than the Mo nitrogenase and contain either vanadium (V) or Fe in place of Mo. Subsequent investigations of other nitrogen-fixing bacteria have shown that these genetically distinct “backup” nitrogenases are widely distributed among nitrogen-fixing bacteria, including *Archaea*, of which a few species fix nitrogen.

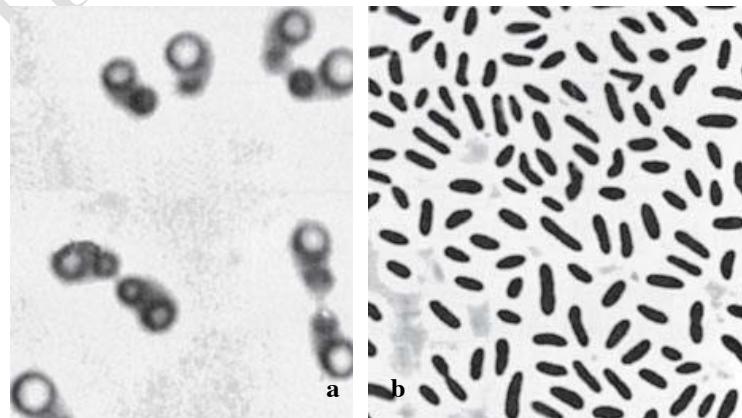


Figure 18: Phase-contrast photomicrographs of two genera of acid-tolerant, free-living N₂-fixing bacteria. (a) *Beijerinckia indica*. The cells are roughly pear-shaped, about 0.8 μm in diameter, and contain a large globule of poly β hydroxybutyrate at each end. (b) *Dexia gummosa*. Cells are about 1 μm in diameter.

1.10 *Neisseria*, *Chromobacterium*, and Relatives

Key Genera: *Neisseria*, *Chromobacterium*

This group of *Beta-* and *Gamma proteobacteria* comprises a diverse collection of organisms that are related phylogenetically as well as by Gram stain, morphology, lack of swimming motility, and aerobic metabolism. The genera *Neisseria*, *Moraxella*, *Branhamella*, *Kingella*, and *Acinetobacter* are distinguished as outlined

in **Table 10**. In the genus *Neisseria*, the cells are always *cocci* (**Figure 19**), whereas cells of the other genera are *rod*-shaped, becoming *coccoid* only in the stationary phase of growth. This has led to designation of these organisms as *coccobacilli*. Organisms of the genera *Neisseria*, *Kingella*, and *Moraxella* are commonly isolated from animals, and some of them are pathogenic. We discuss the clinical microbiology of *Neisseria gonorrhoeae*, the causative agent of the disease gonorrhea, and the pathogenesis of gonorrhea itself. Some *Neisseria* are free-living *saprophytes* and reside in the oral cavity and other moist areas on the animal body, while others, such as *Neisseria meningitidis*, are serious pathogens that can cause a potentially fatal inflammation of the membranes

Table 10 Characteristics of the genera of gram-negative cocci^a

Characteristics	Genus
I. Oxidase-positive, penicillin-sensitive Cocci; complex nutrition, utilize carbohydrates, obligate aerobes; <i>Betaproteobacteria</i> or <i>Gammaproteobacteria</i>	<i>Neisseria</i> <i>Moraxella</i>
Rods or cocci; generally no growth factor requirements, generally do not utilize carbohydrates; do not contain flagella, but some species exhibit twitching motility; many are commensals or pathogens of animals; <i>Betaproteobacteria</i>	<i>Branhamella</i> <i>Kingella</i>
II. Oxidase-negative, penicillin-resistant Some strains can utilize a restricted range of sugars, and some exhibit twitching motility; saprophytes in soil, water, and sewage; <i>Gammaproteobacteria</i>	<i>Acinetobacter</i>

^aAll are *Proteobacteria*.

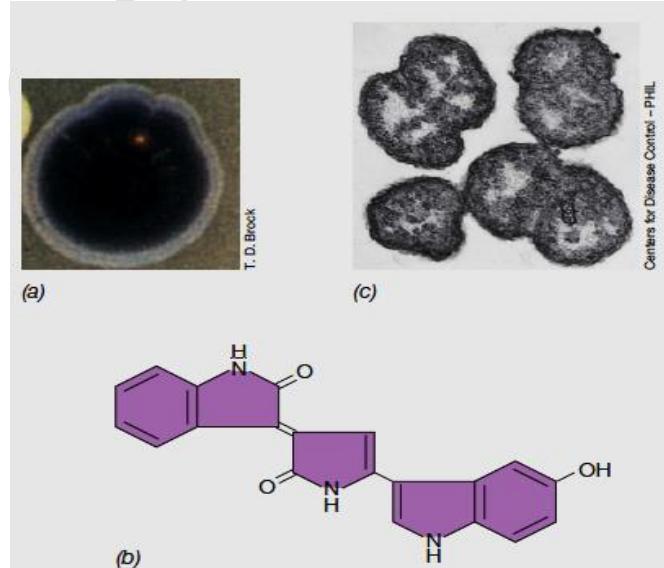


Figure 19: *Chromobacterium* and *Neisseria*. (a) A large colony of *Chromobacterium violaceum*. The purple pigment is an aromatic compound called violacein, the structural formula of which is shown in (b). (c) Transmission electron micrograph of cells of *Neisseria gonorrhoeae* showing the typical diplococcus cell arrangements.

lining the brain (*meningitis*). Species of *Acinetobacter* are common soil and water organisms, although they are occasionally found as *parasites* of animals and have been implicated in some nosocomial (health care associated) infections. Some strains of *Moraxella* and *Acinetobacter* possess the interesting property of *twitching motility*, exhibited as brief translocative movements or “*jumps*” covering distances of about 1–5 μm . Twitching bacteria contain special force generating pili that facilitate their movement.

Chromobacterium is a close phylogenetic relative of *Neisseria* but is *rod*-shaped in morphology, resembling the pseudomonads or enteric bacteria. The best known *Chromobacterium* species is *C. violaceum*, a purple-pigmented organism (Figure 19a) found in soil and water and occasionally in pus-forming infections of humans and other animals. *C. violaceum* and a few other *chromobacteria* produce the purple pigment *violacein* (Figure 19b), a water-insoluble pigment with both antimicrobial and antioxidant properties that is produced only in media containing the amino acid tryptophan, the starting substrate for its synthesis. Like enteric bacteria, *Chromobacterium* is a *facultative aerobe*, growing fermentatively on sugars and aerobically on various carbon sources.

1.11 Enteric Bacteria

Key Genera: *Escherichia*, *Salmonella*, *Proteus*, *Enterobacter*

The **enteric bacteria** comprise a relatively homogeneous phylogenetic group within the *Gammaproteobacteria* and consist of *facultatively aerobic*, gram-negative, nonsporulating rods that are either nonmotile or motile by *peritrichous* flagella (Figure 20). Enteric bacteria are also oxidase-negative, have

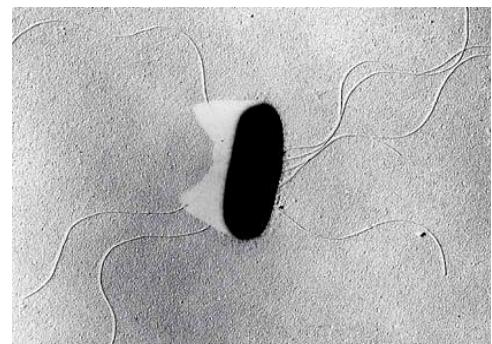


Figure 20: Butanediol producer. Electron micrograph of a shadowcast preparation of cells of the butanediol-producing bacterium *Erwinia carotovora*. The cell is about 0.8 μm wide. Note the peritrichously arranged flagella (arrows), typical of enteric bacteria.

relatively simple nutritional requirements, and ferment sugars to a variety of end products. The defining phenotypic characteristics that distinguish enteric bacteria from other bacteria of similar morphology and physiology are given in **Table 11**. Among the enteric bacteria are many species pathogenic to humans, other animals, or plants, as well as other species of industrial importance. *Escherichia coli*, the best known of all microorganisms, is the classic example of an enteric bacterium. Because of the medical importance of many enteric bacteria, an extremely large number of isolates have been characterized, and numerous genera have been defined, largely for ease in identification purposes in clinical microbiology. However, because enteric bacteria are genetically very closely related, their positive identification often presents considerable difficulty. In clinical laboratories, identification is typically based on the combined analysis of a large number of diagnostic tests carried out using miniaturized rapid diagnostic media kits along with immunological and nucleic acid probes to identify signature proteins or genes of particular species.

Table 11 Defining characteristics of the enteric bacteria

General characteristics

Gram-negative straight rods; motile by peritrichous flagella, or nonmotile; nonsporulating; facultative aerobes, producing acid from glucose; catalase-positive and oxidase-negative; usually reduce NO_3^- to NO_2^- but not to N_2 anaerobically; Gammaproteobacteria (Figure 17.2)

Some major genera

Mixed-acid fermenters: *Escherichia*, *Salmonella*, *Shigella*, *Citrobacter*, *Proteus*, *Yersinia*

Butanediol producers: *Enterobacter*, *Klebsiella*, *Erwinia*, *Serratia*

Key biochemical tests to distinguish enteric bacteria from other bacteria of similar morphology^a

Oxidase test: Enterics always negative—separates enterics from oxidase-positive bacteria of genera *Pseudomonas*, *Aeromonas*, *Vibrio*, *Alcaligenes*, *Achromobacter*, *Flavobacterium*, *Cardiobacterium*, which may have similar morphology

Nitrate reduced only to nitrite (assay for nitrite after growth)—distinguishes enteric bacteria from bacteria that reduce NO_3^- to N_2 (gas formation detected), such as *Pseudomonas* and many other oxidase-positive bacteria

Ability to ferment glucose—distinguishes enterics from obligately aerobic bacteria

Fermentation Patterns in Enteric Bacteria

One major taxonomic characteristic separating the various genera of enteric bacteria is the type and proportion of fermentation products generated from the fermentation of glucose. Two broad patterns are recognized, the mixed-acid fermentation and the 2,3-butanediol fermentation (Table 11 and **Figure 21**). In the mixed-acid fermentation, three acids are formed in significant amounts: acetic, lactic, and succinic; ethanol, CO_2 , and H_2 are also formed, but not butanediol. In

the butanediol fermentation, smaller amounts of acids are formed, and butanediol, ethanol, CO₂, and H₂ are the main products. As a result of mixed-acid fermentation, equal amounts of CO₂ and H₂ are produced, whereas in the butanediol fermentation, considerably more CO₂ than H₂ is produced. This is because mixed acid fermenters produce CO₂ only from formic acid by means of the enzyme system formate hydrogen lyase:

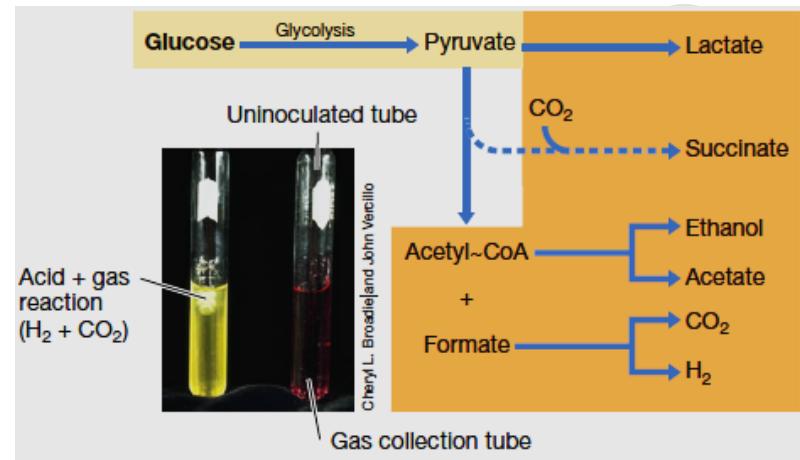


and this reaction results in equal amounts of CO₂ and H₂.

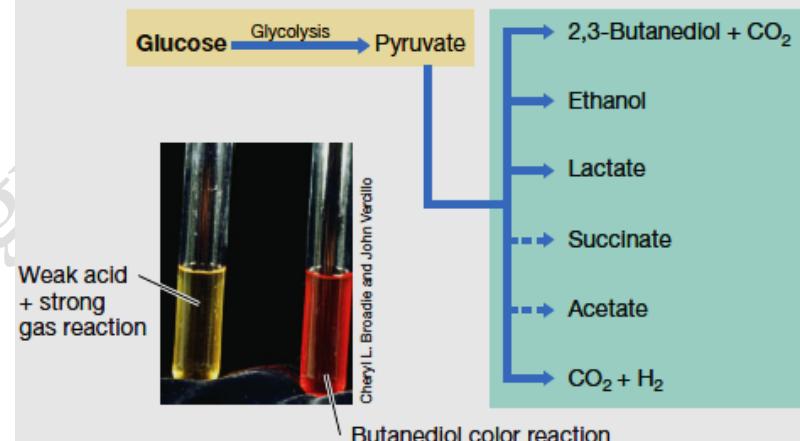
The butanediol fermenters also produce CO₂ and H₂ from formic acid, but they produce two additional molecules of CO₂ during the formation of each molecule of butanediol (Figure 21b).

The Genus *Escherichia*

Species of *Escherichia* are almost universal inhabitants of the intestinal tract of humans and other warm-blooded animals, although they are by no means the dominant



(a) Mixed-acid fermentation (for example, *Escherichia coli*)



(b) Butanediol fermentation (for example, *Enterobacter aerogenes*)

Figure 21: Enteric fermentations. Distinction between (a) mixed acid and (b) butanediol fermentation in enteric bacteria. The solid arrows indicate reactions leading to major products. Dashed arrows indicate minor products. The upper photo shows the production of acid (yellow) and gas (in the inverted Durham tube) in a culture of *Escherichia coli* carrying out a mixed-acid fermentation (purple tube was uninoculated). The bottom photo shows the pink-red color in the Voges –Proskauer (VP) test, which indicates butanediol production, following growth of *Enterobacter aerogenes*. The left (yellow) tube was not inoculated. Note that the mixed-acid fermentation produces less CO₂ but more acid products from glucose than does the butanediol fermentation.

organisms in this habitat. *Escherichia* may play a nutritional role in the intestinal tract by synthesizing vitamins, particularly vitamin K. As a *facultative aerobe*, this organism probably also helps consume O₂, thus rendering the large intestine *anoxic*. Wild-type *Escherichia* strains rarely show any growth-factor requirements and are able to grow on a wide variety of carbon and energy sources such as sugars, amino acids, organic acids, and so on. Some strains of *Escherichia* are pathogenic and have been implicated in diarrheal diseases, especially in infants, a major public health problem in developing countries. *Escherichia* is a major cause of urinary tract infections in women. Enteropathogenic *E. coli* (abbreviated as EPEC) are becoming more frequently implicated in gastrointestinal infections and generalized fevers. certain of these strains form a surface structure called the K antigen, permitting attachment and colonization of the small intestine, and these strains produce enterotoxin, responsible for the signs and symptoms of diarrhea. Some strains, such as enterohemorrhagic *E. coli* (abbreviated as EHEC), an important representative of which is strain O157:H7, can cause sporadic outbreaks of severe foodborne disease. Infection occurs primarily through consumption of contaminated foods, such as raw or undercooked ground beef, unpasteurized milk, or contaminated water. In a small percentage of cases, *E. coli* O157:H7 causes a life-threatening complication related to its production of enterotoxin.

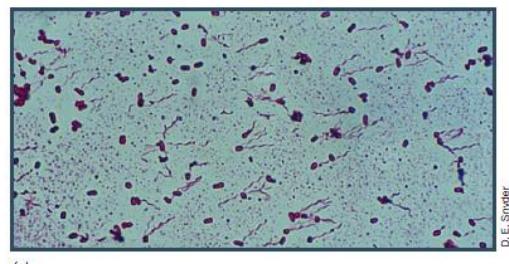
Salmonella, Shigella, and Proteus

Salmonella and *Escherichia* are quite closely related, the two genera showing about 50% genomic hybridization. However, in contrast to most *Escherichia*, species of *Salmonella* are usually pathogenic, either to humans or to other warm-blooded animals; *Salmonella* also is found in the intestines of cold-blooded animals, such as turtles and lizards. In humans the most common diseases caused by salmonellas are typhoid fever and gastroenteritis.

The salmonellas are characterized immunologically on the basis of three cell surface markers called antigens: the O, or cell wall (somatic) antigen; the H, or flagellar, antigen; and the Vi (outer polysaccharide layer) antigen, found primarily in strains of *Salmonella* causing typhoid fever. The O antigens are part of the lipopolysaccharides that constitute the outer membrane of gram-negative bacteria. Although there is little correlation between the antigenic type of *Salmonella* and the disease symptoms elicited, immunological typing permits tracking a single strain type in an epidemic.

The shigellas are also genetically very closely related to *Escherichia*. Tests for DNA hybridization show that strains of *Shigella* have 70% or even higher genomic hybridization with *E. coli* and therefore probably constitute a single species. Moreover, genomic analyses strongly suggest that *Shigella* and *Escherichia* have exchanged a significant number of genes by horizontal gene flow. In contrast to most *Escherichia*, however, species of *Shigella* are typically pathogenic to humans, causing a rather severe gastroenteritis called **bacillary dysentery**. *Shigella dysenteriae*, transmitted by food- and waterborne routes, is a good example of this. The bacterium, which contains endotoxin, invades intestinal epithelial cells, where it excretes a neurotoxin that causes acute gastrointestinal distress.

Cells in the genus *Proteus* are typically highly motile (**Figure 22**) and produce the enzyme urease. By genomic DNA hybridization, *Proteus* shows only a



(a)



(b)

Figure 22: Swarming in *Proteus*. (a) Cells of *Proteus mirabilis* stained with a flagella stain; the peritrichous flagella of each cell form into a bundle to rotate in synchrony. (b) Photo of a swarming colony of *Proteus vulgaris*. Note the concentric rings.

distant relationship to *E. coli*. *Proteus* is a frequent cause of urinary tract infections in humans and probably benefits in this regard from its ready ability to degrade urea. Because of the rapid motility of *Proteus* cells, colonies growing on agar plates often exhibit a characteristic swarming phenotype (Figure 22b). Cells at the edge of the growing colony are more rapidly motile than those in the center of the colony. The former move a short distance away from the colony in a mass and then undergo a reduction in motility, settle down, and divide, forming a new population of motile cells that again swarm. As a result, the mature colony appears as a series of concentric rings, with higher concentrations of cells alternating with lower concentrations (Figure 22b).

Butanediol Fermenters: *Enterobacter*, *Klebsiella*, and *Serratia*

The butanediol fermenters are genetically more closely related to each other than to the mixed-acid fermenters, a finding that is in agreement with the observed physiological differences (Figure 21). *Enterobacter aerogenes* is a common species in water and sewage as well as the intestinal tract of warm-blooded animals and is an occasional cause of urinary tract infections.

One species of *Klebsiella*, *K. pneumoniae*, occasionally causes *pneumonia* in humans, but *klebsiellas* are most commonly found in soil and water. Most *Klebsiella* strains also fix nitrogen, a property unknown in other enteric bacteria.

The genus *Serratia* forms a series of red pyrrole-containing pigments called *prodigiosins* (Figure 23). *Prodigiosin* is produced in stationary phase as a secondary metabolite and is of interest because it contains the pyrrole ring also found in the pigments for energy transfer: porphyrins, chlorophylls, and phycobilins. However, it is unknown if *prodigiosin* plays any role in energy

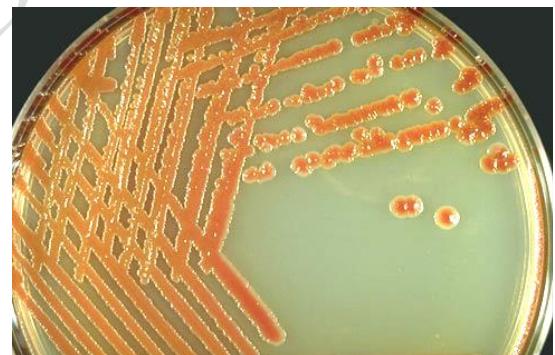


Figure 23: Colonies of *Serratia marcescens*. The orange-red pigmentation is due to the pyrrole-containing pigment prodigiosin.

transfer, and its exact function is unknown. Species of *Serratia* can be isolated from water and soil as well as from the gut of various insects and vertebrates and occasionally from the intestines of humans. *Serratia marcescens* is also a human pathogen that can cause infections in many body sites. It has been implicated in infections caused by some invasive medical procedures and is an occasional contaminant in intravenous fluids.

1.12 *Vibrio*, *Aliivibrio*, and *Photobacterium*

Key Genera: *Vibrio*, *Aliivibrio*, *Photobacterium*

The *Vibrio* group contains gram-negative, *facultatively aerobic rods* and *curved rods* that employ a fermentative metabolism. Most species of *Vibrio* are *polarly flagellated*, although some are *peritrichously* flagellated. One key difference between the *Vibrio* group and enteric bacteria is that members of the former are oxidase-positive, a test for the presence of cytochrome c, whereas members of the latter are oxidase-negative. Although *Pseudomonas* species are also *polarly flagellated* and oxidase-positive, they are not fermentative and so are clearly distinct from *Vibrio* species. The best-known genera in this group are *Vibrio*, *Aliivibrio*, and *Photobacterium*. Most vibrios and related bacteria are aquatic, found in marine, brackish, or freshwater habitats. *Vibrio cholerae* is the specific cause of the disease *cholera* in humans; the organism does not normally cause disease in other hosts. ***Cholera*** is one of the most common human infectious diseases in developing countries and is transmitted almost exclusively via water. *Vibrio parahaemolyticus* inhabits the marine environment and is a major cause of gastroenteritis in Japan, where raw fish is widely consumed; the organism has also been implicated in outbreaks of gastroenteritis in other parts of the world, including the United States. *V. parahaemolyticus* can be isolated from seawater

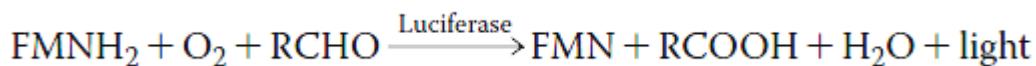
itself or from shellfish and crustaceans, and its primary habitat is probably marine animals, with humans being an accidental host.

Bacterial Bioluminescence

Several species of bacteria can emit light, a process called **bioluminescence** (**Figure 24**). Most bioluminescent bacteria have been classified in the genera *Photobacterium*, *Aliivibrio*, and *Vibrio*, but a few species are found also in *Shewanella*, a genus of primarily marine bacteria, and in *Photorhabdus*, a genus of terrestrial bacteria. Most bioluminescent bacteria inhabit the marine environment and some species colonize specialized light organs of certain marine fishes and squids, producing light that the animal uses for signaling, avoiding predators, and attracting prey (Figure 24c–f). When living symbiotically in light organs of fish and squids, or *saprophytically*, for example, on the skin of a dead fish or *parasitically* in the body of a crustacean, luminous bacteria can be recognized by the light they produce. Because some pathogenic strains of *Vibrio* are also luminous, such as certain strains of *V. cholerae* and *V. vulnificus*, care should always be taken when isolating and handling luminous bacteria.

Mechanism and Ecology of Bioluminescence

Although *Photobacterium*, *Aliivibrio*, and *Vibrio* isolates are *facultative aerobes*, they are bioluminescent only when O₂ is present. Luminescence in bacteria requires the genes *luxCDABE* and is catalyzed by the *enzyme luciferase*, which uses O₂, a long-chain aliphatic aldehyde such as tetradecanal, and reduced flavin mononucleotide (FMNH₂) as substrates:



The light-generating system constitutes a metabolic route for shunting electrons from FMNH₂ to O₂ directly, without employing other electron carriers such as quinones and cytochromes. Luminescence in many luminous bacteria only occurs

at high population density. The enzyme *luciferase* and other proteins of the bacterial luminescence system exhibit a population density–responsive induction, called **autoinduction**, in which transcription of the *luxCDABE* genes is controlled by a regulatory protein, LuxR, and an inducer molecule, acyl homoserine lactone (AHL). During growth, cells produce AHL, which can rapidly cross the cytoplasmic membrane in either direction, diffusing in and out of cells. Under conditions in which a high local population density of cells is attained, as in a test tube, a colony on a plate, or in the light organ of a fish or squid, AHL accumulates. Only when it reaches a certain concentration in the cell is it bound by LuxR, forming a complex that activates transcription of *luxCDABE*, and cells become luminous (Figure 24b). This gene regulatory mechanism is also called *quorum sensing* because of the population density–dependent nature of the phenomenon.

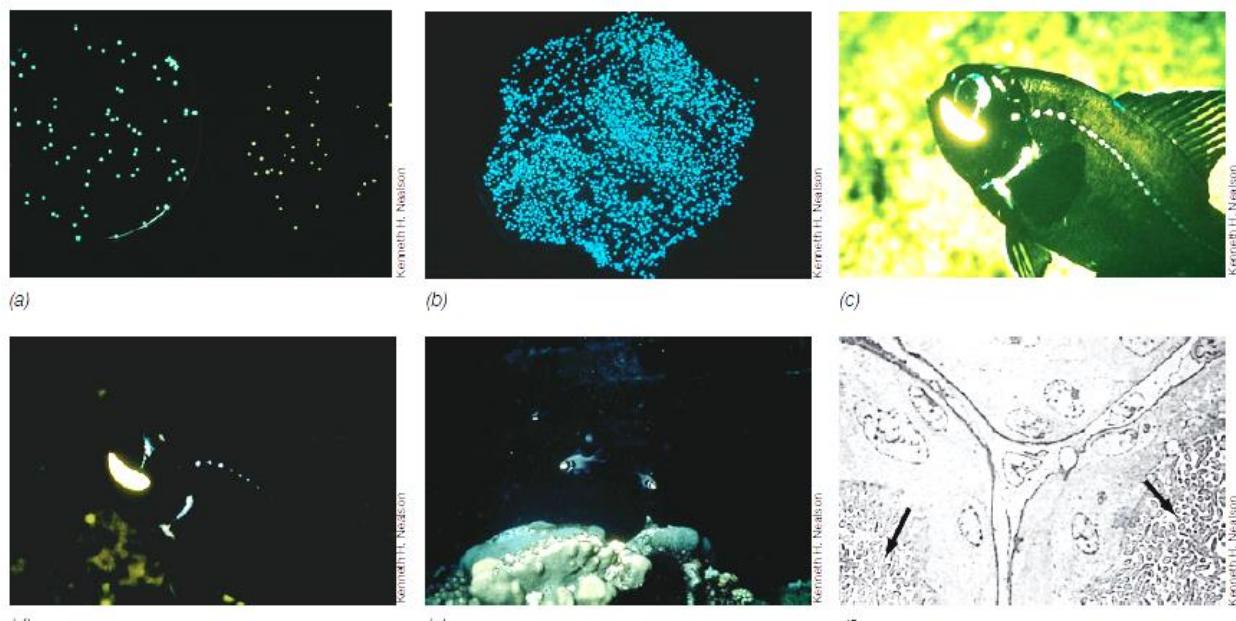


Figure 24: Bioluminescent bacteria and their role as light organ symbionts in the flashlight fish. (a) Two Petri plates of luminous bacteria photographed by their own light. Note the different colors. Left, *Aliivibrio fischeri* strain MJ-1, blue light, and right, strain Y-1, green light. (b) Colonies of *Photobacterium phosphoreum* photographed by their own light. (c) The flashlight fish *Photoblepharon palpebratus*; the bright area is the light organ containing bioluminescent bacteria. (d) Same fish photographed by its own light. (e) Underwater photograph taken at night of *P. palpebratus*. (f) Electron micrograph of a thin section through the light-emitting organ of *P. palpebratus* showing the dense array of bioluminescent bacteria (arrows).

In saprophytic, parasitic, and symbiotic habitats (Figure 24c–f), the rationale for population density–responsive induction of luminescence is to ensure that luminescence develops only when sufficiently high population densities are reached to allow the light produced to be visible to animals. The bacterial light can then attract animals to feed on the luminous material, thereby bringing the bacteria into the animal’s nutrient-rich gut for further growth. Alternatively, the luminous material may function as a light source in symbiotic, light organ associations. Quorum sensing is a form of regulation that has also been found in many different nonluminous bacteria, including several animal and plant pathogens. Quorum sensing in these bacteria controls activities such as the production of extracellular enzymes and expression of virulence factors for which a high population density is beneficial if the bacteria are to have a biological effect.

1.13 Rickettsias

Key Genera: *Rickettsia*, *Wolbachia*

The rickettsias are small, gram-negative, coccoid or rod-shaped *Alpha-* or *Gamma proteobacteria* in the size range of 0.3–0.7 X 1–2 μm . They are, with one exception, obligate intracellular parasites and have not yet been cultivated in the absence of host cells (Figure 25). Rickettsias are the causative agents of several human diseases, including typhus, Rocky Mountain spotted fever, and Q fever. Electron micrographs of

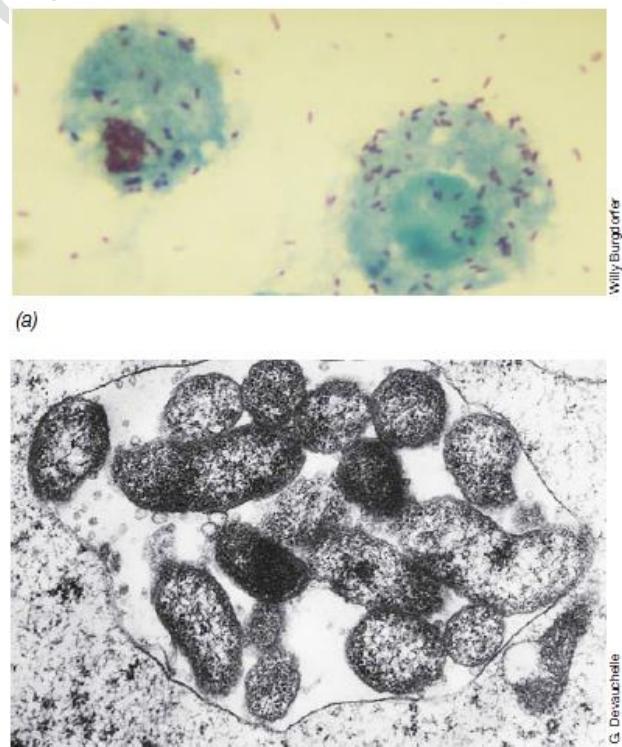


Figure 25: Rickettsias growing within host cells. (a) *Rickettsia rickettsii* in tissue culture. Cells are about 0.3 μm in diameter. (b) Electron micrograph of cells of *Rickettsiella popilliae* within a blood cell of its host, the beetle *Melolontha melolontha*. The bacteria grow inside a vacuole within the host cell.

thin sections of rickettsial cells show a typical *prokaryotic* morphology (Figure 25b); both cell wall and cytoplasmic membrane are clearly present. The rickettsial cell wall contains peptidoglycan, and the cells divide by binary fission. The penetration of a host cell by a rickettsial cell is an active process, requiring both host and parasite to be alive and metabolically active. Once inside the host cell, the bacteria multiply primarily in the cytoplasm and continue replicating until the host cell is loaded with parasites (Figure 25). The host cell then bursts and liberates the bacterial cells. Several genera of rickettsias are known, and the properties of five key genera are shown in **Table 12**.

Table 12 Some characteristics of rickettsias

Genus and species	Rickettsial group	Alternate host	Cellular location	Phylogenetic group ^a	DNA hybridization to R. rickettsii DNA (%) ^b
Rickettsia					
<i>R. rickettsii</i>	Spotted fever	Tick	Cytoplasm and nucleus	Alpha	100
<i>R. prowazekii</i> ^c	Typhus	Louse	Cytoplasm	Alpha	53
<i>R. typhi</i>	Typhus	Flea	Cytoplasm	Alpha	36
Rochalimaeae					
<i>R. quintana</i>	Trench fever	Louse	Epicellular	Alpha	30
<i>R. vinsonii</i>	—	Vole	Epicellular	Alpha	30
Coxiella					
<i>C. burnetii</i>	Q fever	Tick	Vacuoles	Gamma	—
Ehrlichia					
<i>E. chaffeensis</i>	Ehrlichiosis (humans)	Tick or domestic animals	Mononuclear leukocytes	Alpha	—
<i>E. equi</i>	Potomac fever (horses)	Tick	Granulocyte	Alpha	—
Wolbachia^d					
<i>W. pipiensis</i>	—	Arthropods	Cytoplasm	Alpha	—

^aAll are Proteobacteria.

Metabolism and Pathogenesis

Most rickettsias (an exception is *Coxiella burnetii*, the causative agent of the disease Q fever) possess a highly specific energy metabolism: They oxidize only the amino acids glutamate or glutamine and cannot oxidize glucose or organic acids. Rickettsias synthesize a respiratory chain complete with cytochromes and are able to carry out electron transport phosphorylation using NADH as electron donor. They are also able to synthesize at least some of the small molecules needed

for macromolecular synthesis and growth, but obtain the rest of their nutrients from the host cell. Thus, although parasites, rickettsias maintain a number of independent metabolic functions. Rickettsias do not survive long outside their hosts, and this may explain why they must be transmitted from animal to animal by arthropod vectors. When the arthropod obtains a blood meal from an infected animal, rickettsias present in the blood are ingested and penetrate the epithelial cells of the gastrointestinal tract; there they multiply and appear later in the feces. When the arthropod feeds on an uninfected individual, it then transmits the rickettsias either directly with its mouthparts or by contaminating the bite with its feces. *C. burnetii* can also be transmitted person-to-person in infectious aerosols. Other pathogenic rickettsias include the genera *Rochalimaea* and *Ehrlichia*. *Rochalimaea* is an atypical rickettsia because it can be grown in culture and is thus not an obligate intracellular parasite. In addition, when growing in tissue culture, cells of *Rochalimaea* grow on the outside surface of the *eukaryotic* host cells rather than within the cytoplasm or the nucleus as do other rickettsias. *Rochalimaea quintana* is the causative agent of **trench fever**, a disease that decimated troops in World War I. Species of the genus *Ehrlichia* cause disease in humans and other animals. Two of these diseases, **ehrlichiosis** in humans and **Potomac fever** in horses, can be quite debilitating.

Wolbachia

The genus *Wolbachia* contains species of rod-shaped *Alphaproteobacteria* that are intracellular parasites of several families of insects, a huge group that constitutes 70% of all known arthropod species (**Figure 26**). *Wolbachia* are phylogenetically related to the rickettsias and can have any of several effects on their insect hosts. These include inducing parthenogenesis (development of unfertilized eggs), the killing of males, and feminization (the conversion of male

insects into females). *Wolbachia pipiensis* is the best-studied species in the genus. *W. pipiensis* has a relatively small genome (about 1.5 Mbp), which is actually quite large by insect symbiont standards. Cells of *W. pipiensis* colonize the insect egg (Figure 26), where they multiply in vacuoles of host cells surrounded by a membrane of host origin. Cells of *W. pipiensis* are passed from an infected female to her offspring through this egg infection.

Wolbachia-induced parthenogenesis occurs in a number of species of wasps. In these insects, males normally arise from unfertilized eggs (which contain only one set of chromosomes), while females arise from fertilized eggs (which contain two sets of chromosomes). However, in unfertilized eggs infected with *Wolbachia*, the organism somehow triggers a doubling of the chromosome number, thus yielding only females. Predictably, if female insects are fed antibiotics that kill *Wolbachia*, parthenogenesis ceases.



Figure 26: *Wolbachia*. Photomicrograph of a DAPI-stained egg of the parasitoid wasp *Trichogramma kaykai* infected with *Wolbachia pipiensis*, which induces parthenogenesis. The *W. pipiensis* cells are primarily located in the narrow end of the egg (arrows).

IV- Morphologically Unusual *Proteobacteria*

Some *Proteobacteria* have unusual morphologies or undergo fascinating life cycles. We consider some of these here, focusing on common curved and spiral-shaped bacteria, a few filamentous bacteria that encase their cells in a sheath, and the morphologically unusual prosthecate and stalked bacteria. In the latter group, we will focus on the important model bacterium for the study of cell differentiation, *Caulobacter*. Physiologically, all of these organisms are *chemoorganotrophs*, consuming organic matter in a wide variety of primarily aquatic habitats.

1.14 Spirilla

Key Genera: *Spirillum*, *Magnetospirillum*, *Bdellovibrio*

The **spirilla** are gram-negative, motile, *spiral*-shaped bacteria that show a wide variety of physiological attributes. Some key taxonomic criteria used are cell shape, size, and number of flagella, relation to oxygen (*obligately aerobic*, *microaerophilic*), relationship to higher organisms, and certain other physiological characteristics, such as nitrogen fixation and *halophilism*. The genera to be covered here are given in **Table 13**, where it can be seen that spirilla are found in each of the five classes of *Proteobacteria* (the genera *Campylobacter* and *Helicobacter* are *Epsilonproteobacteria*).

Spirillum, *Aquaspirillum*, *Oceanospirillum*, and *Azospirillum*

The spirilla, which are helically curved rods, are motile by means of *polar* flagella, usually tufts at both poles (**Figure 27**). The number of turns in the helix may vary from less than one complete turn (in which case the organism looks like a vibrio) to many turns. Spirilla with many turns can superficially resemble *spirochetes* but differ distinctly from the latter phylogenetically. In addition,

spirilla do not have the outer sheath and *endoflagella* of *spirochetes*, but instead contain typical bacterial flagella.

The cells of some spirilla are very large and are easily observed. For example, *Spirillum volutans*, a large *spirillum*, is common in aquatic environments and is *microaerophilic*, requiring O₂ but inhibited by O₂ at normal atmospheric levels. Another prominent characteristic of cells of *S. volutans* is the formation of intracellular inclusions called *volutin granules*, consisting of polyphosphate (Figure 27a).

Table 13 Characteristics of some major genera of spiral-shaped bacteria ^a		
Genus	Phylogenetic group ^b	Characteristics
<i>Spirillum</i>	Beta	Cell diameter 1.7 µm; microaerophilic; freshwater
<i>Aquaspirillum</i>	Beta	Cell diameter 0.2–1.5 µm; aerobic; freshwater
<i>Magnetospirillum</i>	Alpha	Vibrio to spirillum-shaped; cell diameter about 0.3 µm; contains magnetosomes; microaerophilic
<i>Oceanospirillum</i>	Gamma	Cell diameter 0.3–1.2 µm; aerobic; marine (require 3% NaCl)
<i>Azospirillum</i>	Alpha	Cell diameter 1 µm; microaerophilic; soil and rhizosphere; fixes N ₂
<i>Herbaspirillum</i>	Beta	Cell diameter 0.6–0.7 µm; microaerophilic; soil and rhizosphere; fixes N ₂
<i>Bdellovibrio</i>	Delta	Cell diameter 0.25–0.4 µm; aerobic; predatory on other bacteria; single polar sheathed flagellum
<i>Ancylobacter</i>	Alpha	Cell diameter 0.5 µm; curved rods forming rings; nonmotile, aerobic; sometimes gas vesiculate

^aAll are gram-negative and respiratory, but never fermentative.

^bAll are *Proteobacteria*.

Azospirillum lipoferum is a nitrogen-fixing organism and of considerable interest because it enters into a symbiotic relationship with tropical grasses and grain crops such as corn. Although not the intimate association that forms between root nodule bacteria of the genus *Rhizobium* and leguminous plants, the *A. lipoferum*–corn association clearly benefits the corn plant by fixed nitrogen supplied by nitrogen fixation.

The small-diameter spirilla are fully *aerobic* and have been separated into two genera, *Aquaspirillum* and *Oceanospirillum*. The former includes freshwater species and the latter includes species that inhabit seawater and require sodium chloride (NaCl) for growth (Table 13). Numerous species of *Aquaspirillum* and *Oceanospirillum* have been described, and the various species are separated on

physiological and phylogenetic grounds. These organisms undoubtedly play an important role in the recycling of organic matter in *oxic* aquatic environments.

Magnetotactic Spirilla

Highly motile, *microaerophilic*, magnetic spirilla have been isolated from freshwater habitats. These organisms demonstrate a dramatic directed movement in a magnetic field called **magnetotaxis**. The *spirillum* *Magnetospirillum magnetotacticum* (**Figure 28** and Table 13) is a major organism in this group. In an artificial magnetic field, magnetic spirilla quickly orient their long axis along the north–south magnetic moment of the field. Within the cells are chains of magnetic particles called **magnetosomes**, consisting of the iron minerals magnetite (Fe_3O_4) and greigite (Fe_3S_4). Magnetic bacteria display one of two magnetic polarities depending on the orientation of magnetosomes within the cell. Cells in the Northern Hemisphere have the north-

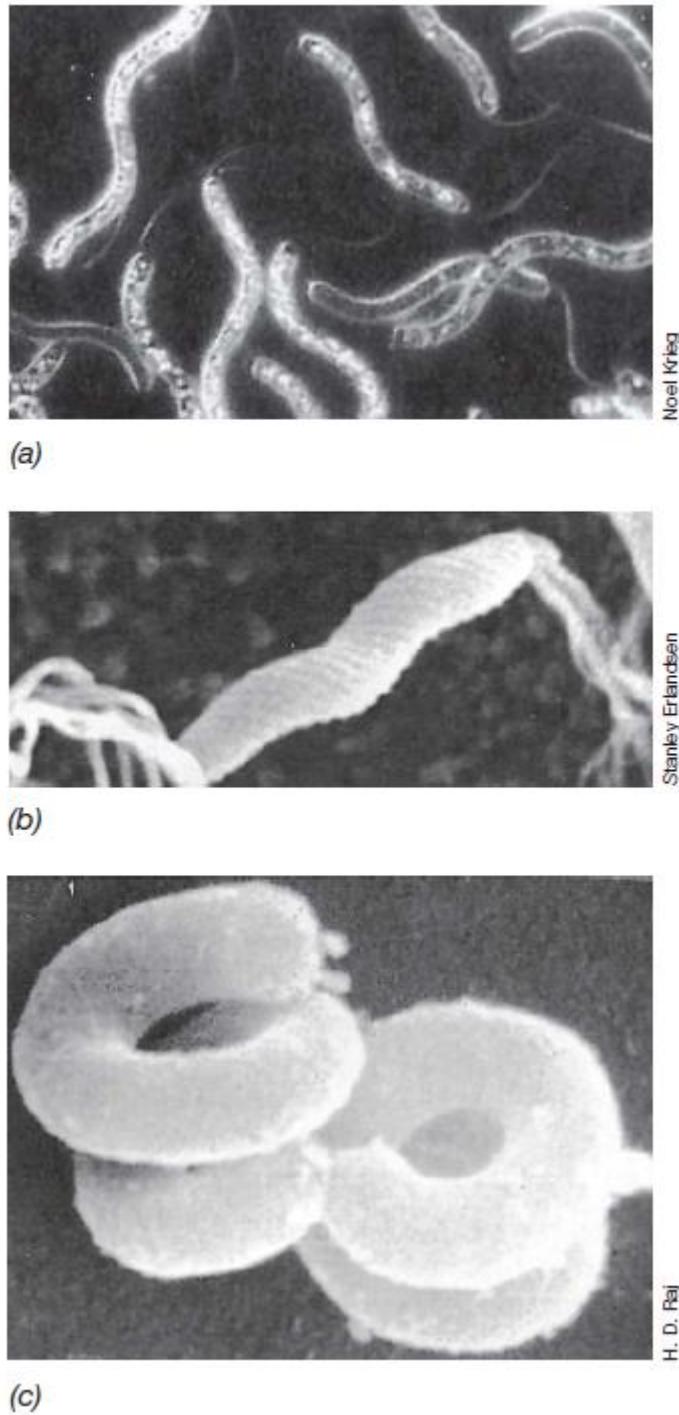


Figure 27: Spirilla. (a) *Spirillum volutans*, visualized by dark-field microscopy, showing flagellar bundles and volutin (polyphosphate) granules. Cells are about $1.5 \times 25 \mu\text{m}$. (b) Scanning electron micrograph of an intestinal spirillum. Note the polar flagellar tufts and the spiral structure of the cell surface. (c) Scanning electron micrograph of cells of *Ancylobacter aquaticus*. Cells are about $0.5 \mu\text{m}$ in diameter.

seeking pole of their magnetosomes forward with respect to their flagella and thus move in a northward direction. Cells in the Southern Hemisphere have the opposite polarity and move southward. Although the ecological role of bacterial magnets is unclear, the ability to orient in a magnetic field may be of selective advantage in maintaining these *microaerophilic* organisms in zones of low O₂ concentration near the *oxic-anoxic* interface.

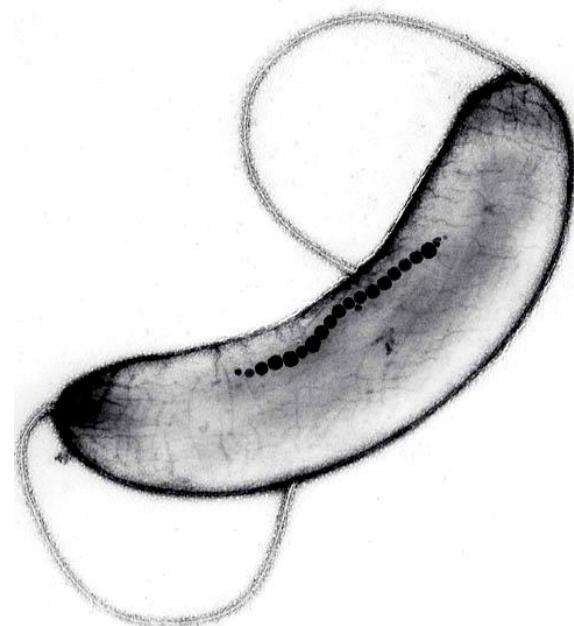


Figure 28: Magnetotactic spirillum. Electron micrograph of a single cell of *Magnetospirillum magnetotacticum*; a cell measures 0.3 X 2 μm . The cell contains particles of magnetosomes made of Fe₃O₄ arranged in a chain..

Bdellovibrio

Bdellovibrio is a genus of small, highly motile and curved bacteria that prey on other bacteria, using the cytoplasmic constituents of their hosts as nutrients (bdello is a prefix meaning “leech”). After attachment of a *Bdellovibrio* cell to its prey, the predator penetrates the cell wall of the prey and replicates in the periplasmic space, eventually forming a spherical structure called a *bdelloplast*. Two stages of

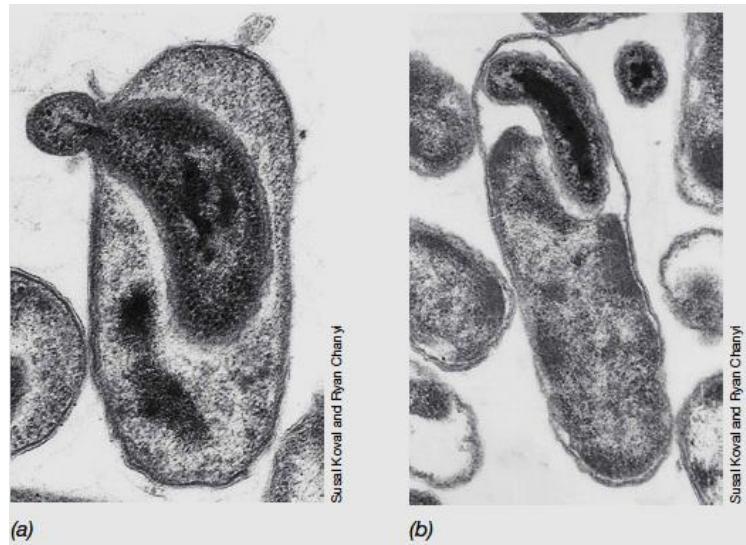


Figure 29: Attack on a prey cell by *Bdellovibrio*. Thin-section electron micrographs of *Bdellovibrio* attacking a cell of *Delftia* (formerly *Comamonas*) *acidovorans*. (a) Entry of the predator cell. (b) *Bdellovibrio* cell inside the host. The *Bdellovibrio* cell is enclosed in the *bdelloplast* and replicates in the periplasmic space. A *Bdellovibrio* cell measures about 0.3 μm in diameter.

penetration are shown in electron micrographs in **Figure 29** and diagrammatically in **Figure 30**. A wide variety of gram-negative prey bacteria can be attacked by *Bdellovibrio*, but gram-positive cells are not attacked. *Bdellovibrio* is an *obligate aerobe*, obtaining its energy from the oxidation of amino acids and acetate. In addition, *Bdellovibrio* assimilates nucleotides, fatty acids, peptides, and even some intact proteins directly from its host without first breaking them down. Prey-independent derivatives of predatory strains of *Bdellovibrio* can be isolated and grown on complex media, however, showing that predation is not obligatory. Phylogenetically, bdellovibrios are species of *Deltaproteobacteria* and are widespread in soil and aquatic habitats. Procedures for their isolation are similar to those used to isolate bacterial viruses. Prey bacteria are spread on the surface of an agar plate to form a lawn and the surface is inoculated with a small amount of soil suspension that has been filtered through a membrane filter; the filter retains most bacteria, but allows the small *Bdellovibrio* cells to

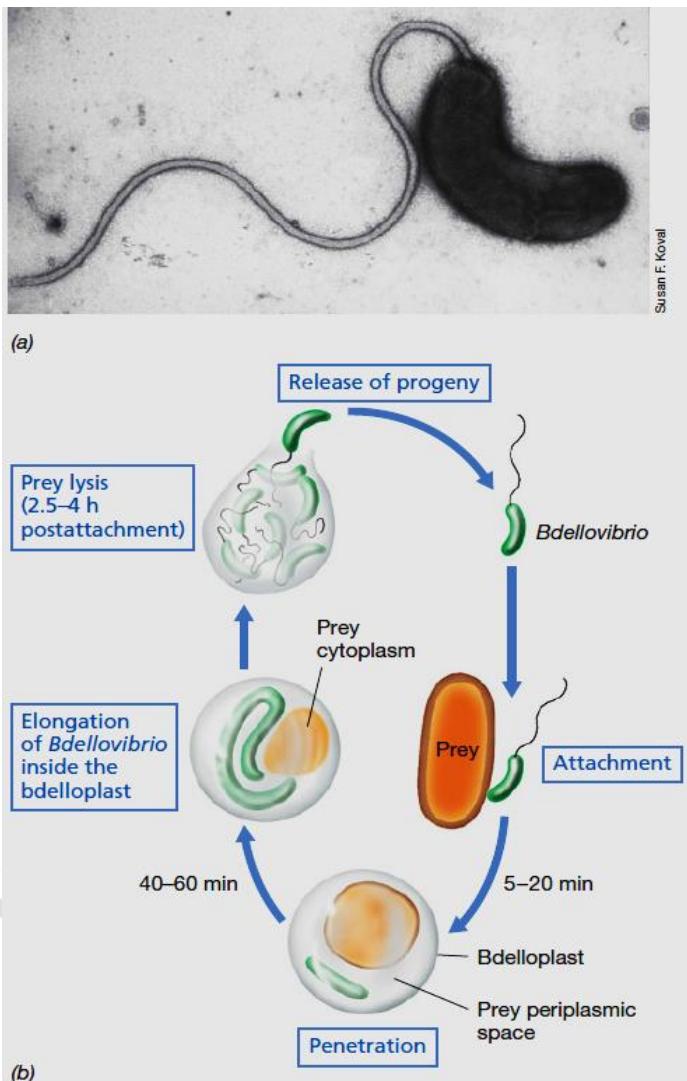


Figure 30: Developmental cycle of the bacterial predator *Bdellovibrio bacteriovorus*. (a) Electron micrograph of a cell of *Bdellovibrio bacteriovorus*. Note the very thick flagellum. (b) Events in predation. Following primary contact with a gram-negative bacterium, the highly motile *Bdellovibrio* cell attaches to and penetrates into the prey periplasmic space. Once inside the periplasmic space, *Bdellovibrio* cells elongate and within 4 h progeny cells are released. The number of progeny cells released varies with the size of the prey bacterium. For example, 5–6 *bdellovibrios* are released from each infected *Escherichia coli* cell and 20–30 for a larger cell, such as a species of *Aquaspirillum*.

pass. On incubation of the agar plate, plaques analogous to bacteriophage plaques are formed at locations where *Bdellovibrio* cells are growing. Pure cultures of *Bdellovibrio* can then be isolated from these plaques. *Bdellovibrio* cultures have been obtained from many soils and are thus widespread in distribution.

Ancylobacter

Species of *Ancylobacter* are ring-shaped, nonmotile, extremely diverse nutritionally, *aerobic* and *chemoorganotrophic* bacteria (Figure 27c). They resemble very tightly coiled vibrios and are widely distributed in aquatic environments. A *phototrophic* counterpart to *Ancylobacter* is the purple nonsulfur bacterium *Rhodocyclus purpureus*, considered earlier.

1.15 Sheathed Proteobacteria: *Sphaerotilus* and *Leptothrix*

Key Genera: *Sphaerotilus*, *Leptothrix*

Sheathed bacteria are filamentous *Betaproteobacteria* (Figure 1) with a unique life cycle in which flagellated swarmer cells form within a long tube or sheath. Under unfavorable growth conditions, the swarmer cells move out and become dispersed to new environments, leaving behind the empty sheath. Under favorable conditions, the cells grow vegetatively within the sheath, leading to the formation of long, cell-packed sheaths. Sheathed bacteria are common in freshwater habitats that are rich in organic matter, such as wastewaters and polluted streams. Because they are typically found in flowing waters, they are also abundant in trickling filters and activated sludge digesters in sewage treatment plants. In habitats in which reduced iron (Fe^{2+}) or manganese (Mn^{2+}) is present, the sheaths may become coated with ferric hydroxide Fe(OH)_3 or various manganese oxides from the oxidation of these metals.

Sphaerotilus

The *Sphaerotilus* filament is composed of a chain of rod-shaped cells enclosed in a closely fitting sheath. This thin, transparent structure is difficult to see when it is filled with cells, but when it is partially empty, the sheath can more easily be seen. Individual cells are 1–2 µm wide and 3–8 µm long and stain gram negatively. The cells within the sheath divide by binary fission, and the new cells synthesize new sheath material at the tips of the filaments. Eventually, motile swarmer cells are liberated from the sheaths that then migrate, attach to a solid surface, and begin to grow, with each swarmer being the forerunner of a new filament. The sheath, which is devoid of peptidoglycan, consists of protein and polysaccharide.

Sphaerotilus cultures are nutritionally versatile and use simple organic compounds as carbon and energy sources. Befitting its habitat in flowing waters, *Sphaerotilus* is an *obligate aerobe*. Large masses (blooms) of *Sphaerotilus* often occur in the fall of the year in streams and brooks when leaf litter causes a temporary increase in the organic content of the water. In addition, its filaments are the main component of a microbial complex that wastewater engineers call “sewage fungus,” a filamentous slime found on the rocks in streams receiving sewage pollution. In activated sludge of sewage treatment plants, *Sphaerotilus* is often responsible for a condition called ***bulking***, where the tangled masses of *Sphaerotilus* filaments so increase the bulk of the sludge that it remains suspended and does not settle as it should. This has a negative effect on the oxidation of organic matter and the recycling of inorganic nutrients and leads to treatment plant discharges with high nitrogen and carbon loads.

Leptothrix

The ability of *Sphaerotilus* and *Leptothrix* to precipitate iron oxides on their sheaths is well established, and when sheaths become iron encrusted, as occurs in iron-rich waters, they can frequently be seen microscopically (Figure 31). Iron precipitates when ferrous iron (Fe^{2+}), chelated to organic materials such as humic or tannic acids, is oxidized to iron oxides. Fe^{2+} binds to the sheath, and the organic constituents are taken up and used as a carbon or energy source. Iron oxidation is fortuitous and the organism does not use Fe^{2+} as an electron donor in energy metabolism as many iron-oxidizing bacteria do. Besides Fe^{2+} oxidation, *Leptothrix* can also oxidize manganese, typically Mn^{2+} containing minerals to Mn^{4+} containing minerals, but as for Fe^{2+} , Mn^{2+} oxidation does not yield energy for the organism.

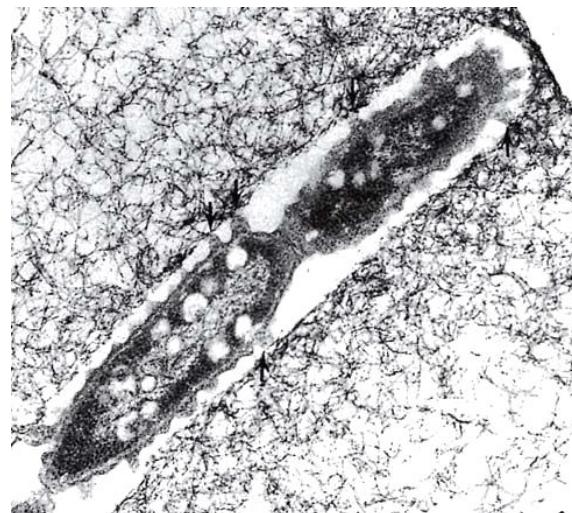


Figure 31: *Leptothrix* and iron precipitation. Transmission electron micrograph of a thin section of *Leptothrix* in a sample from a ferromanganese film in a swamp in Ithaca, New York. A single cell measures about $0.9 \mu\text{m}$ in diameter. Note the protuberances of the cell envelope that contact the sheath (arrows).

1.16 Budding and Prosthecate/ Stalked Bacteria

Key Genera: *Hyphomicrobium*, *Caulobacter*

This large and heterogeneous group of primarily *Alphaproteobacteria* contains organisms that form various kinds of cytoplasmic extrusions: stalks, hyphae, or appendages (Table 14). Extrusions of these kinds, which are smaller in diameter than the mature cell and contain cytoplasm and a cell wall, are collectively called **prosthecae** (Figure 32).

Budding Division

Budding bacteria divide as a result of unequal cell growth. In contrast to binary fission that forms two equivalent cells, cell division in stalked and budding

bacteria forms a totally new daughter cell, with the mother cell retaining its original identity (**Figure 33**). A fundamental difference between these bacteria and bacteria that divide by binary fission is the formation of new cell wall material from a single point (polar growth) rather than throughout the whole cell (intercalary growth) as in binary fission.

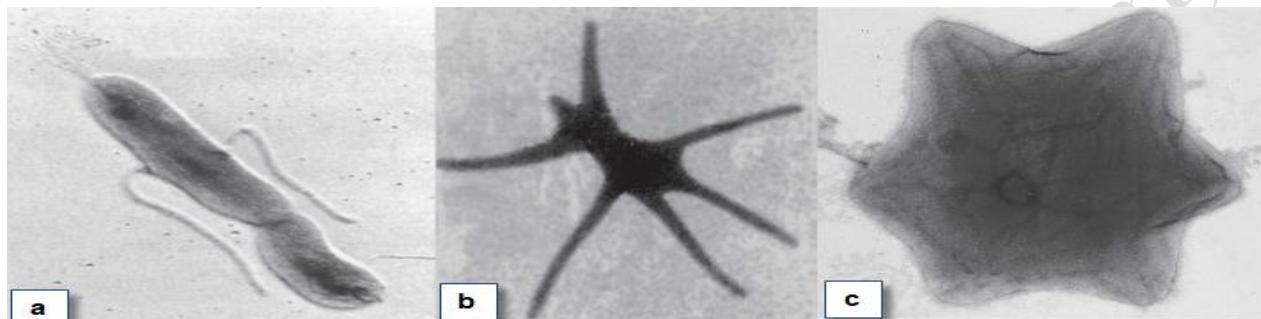


Figure 32: Prosthecate bacteria. (a) Electron micrograph of a shadow-cast preparation of *Asticcacaulis biprosthecum*, illustrating the location and arrangement of the prosthocysts, the holdfast, and swarmer cell. The swarmer cell breaks away from the mother cell and begins a new cell cycle. Cells are about 0.6 µm wide. (b) Negatively stained electron micrograph of a cell of *Ancalomicrombium adetum*. The prosthocysts are bounded by the cell wall, contain cytoplasm, and are about 0.2 µm in diameter. (c) Electron micrograph of the star-shaped bacterium *Stella*. Cells are about 0.8 µm in diameter.

Table 14 | Characteristics of major genera of stalked, appendaged (prosthecate), and budding bacteria

Characteristics	Genus	Phylogenetic group ^a
Stalked bacteria		
Stalk an extension of the cytoplasm and involved in cell division	<i>Caulobacter</i>	Alpha
Stalked, fusiform-shaped cells	<i>Prosthecobacter</i>	Verrucomicrobiaceae ^b
Stalked, but stalk is an excretory product not containing cytoplasm:		
Stalk depositing iron, cell vibrioid	<i>Gallionella</i>	Beta
Laterally excreted gelatinous stalk not depositing iron	<i>Nevskia</i>	Gamma
Appendaged (prosthecate) bacteria		
Single or double prosthocysts	<i>Asticcacaulis</i>	Alpha
Multiple prosthocysts:		
Short prosthocysts, multiply by fission, some with gas vesicles	<i>Prosthecomicrobium</i>	Alpha
Flat, star-shaped cells, some with gas vesicles	<i>Stella</i>	Alpha
Long prosthocysts, multiply by budding, some with gas vesicles	<i>Ancalomicrombium</i>	Alpha
Budding bacteria		
Phototrophic, produce hyphae	<i>Rhodomicrombium</i>	Alpha
Phototrophic, budding without hyphae	<i>Rhodopseudomonas</i>	Alpha
Chemoorganotrophic, rod-shaped cells	<i>Blastobacter</i>	Alpha
Chemoorganotrophic, buds on tips of slender hyphae:		
Single hyphae from parent cell	<i>Hyphomicrobium</i>	Alpha
Multiple hyphae from parent cell	<i>Pedomicrombium</i>	Alpha

^aAll but *Prosthecobacter* are Proteobacteria.

Several genera not normally considered to be budding bacteria show polar growth without differentiation of cell size (Figure 33). An important consequence

of polar growth is that internal structures, such as membrane complexes, are not partitioned in the cell division process and must be formed de novo. However, this has an advantage in that more complex internal structures can be formed in budding cells than in cells that divide by binary fission, since the latter cells would have to partition these structures between the two newly forming daughter cells. Not coincidentally, many budding bacteria, particularly *phototrophic* and *chemolithotrophic* species, contain extensive internal membrane systems.

Budding Bacteria: *Hyphomicrobium*

Two well-studied budding bacteria are closely related phylogenetically: *Hyphomicrobium*, which is *chemoorganotrophic*, and *Rhodomicrobiun*, which is *phototrophic*. These organisms release buds from the ends of long, thin hyphae. The hypha is a direct cellular extension and contains cell wall, cytoplasmic membrane, and ribosomes, and can contain DNA. **Figure 34** shows the life cycle of *Hyphomicrobium*. The mother cell, which is often attached by its base to a solid substrate, forms a thin outgrowth that lengthens to become a hypha. At the end of the hypha, a bud forms. This bud enlarges, forms a flagellum, breaks loose from the mother cell, and swims away. Later, the daughter cell loses its flagellum and after a period of maturation forms a hypha and buds. More buds can also form at the hyphal tip of the mother cell, leading to arrays of cells connected by hyphae. In some cases, a bud begins to form directly from the mother cell without the intervening formation of a hypha, whereas in other cases a single cell forms hyphae from each end (**Figure 35**).

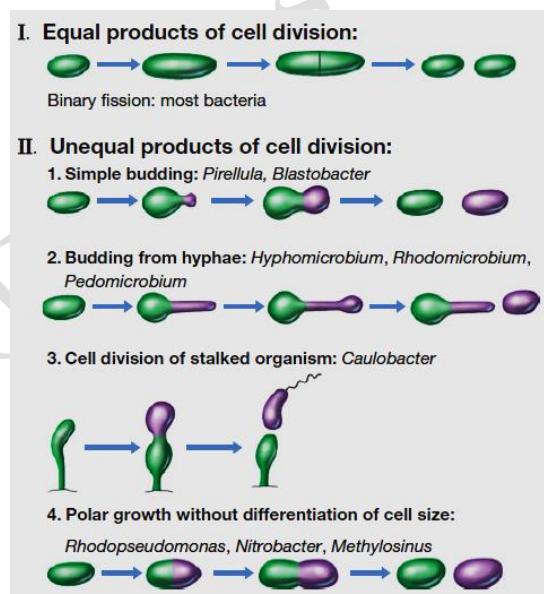


Figure 33: Cell division in different bacteria. Contrast between cell division in conventional bacteria and in various budding and stalked bacteria.

Nucleoid replication events occur before the bud emerges, and then once a bud has formed, a copy of the chromosome moves down the hypha and into the bud. A cross septum then forms, separating the still-developing bud from the hypha and mother cell (Figure 34). Physiologically, *Hyphomicrobium* is a methylotrophic bacterium, and it is widespread in freshwater, marine, and terrestrial habitats. Preferred carbon sources are C₁ compounds such as methanol (CH₃OH), methylamine (CH₃NH₂), formaldehyde (CH₂O), and formate (HCOO⁻). A fairly specific enrichment procedure for *Hyphomicrobium* uses CH₃OH as electron donor with nitrate (NO₃⁻) as electron acceptor in a dilute medium incubated under *anoxic* conditions. The only rapidly growing denitrifying bacterium known that uses CH₃OH as electron donor is *Hyphomicrobium* and so this procedure can select this organism out of a wide variety of environments.

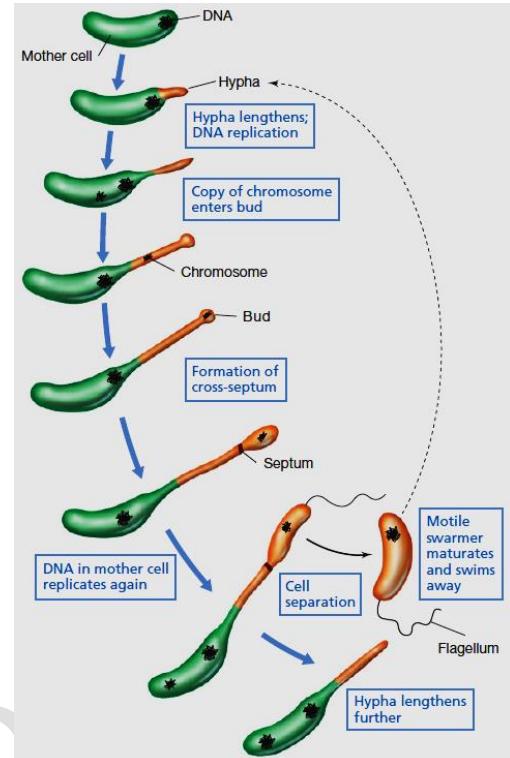


Figure 34: Stages in the *Hyphomicrobium* cell cycle. The single chromosome of *Hyphomicrobium* is circular.

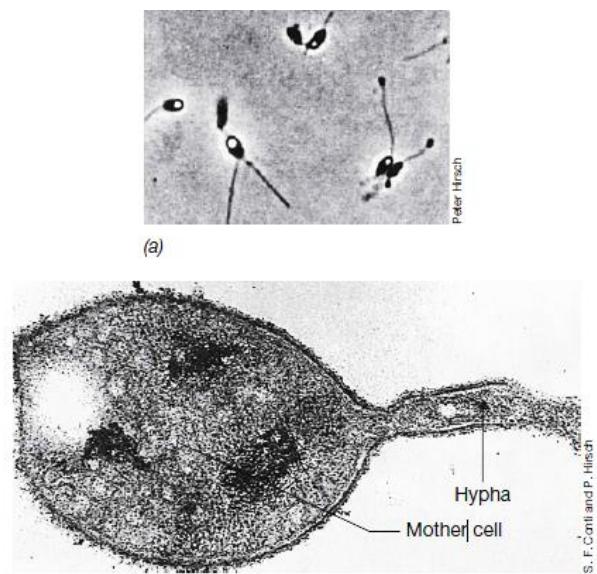


Figure 35: Morphology of *Hyphomicrobium*. (a) Phase-contrast micrograph of cells of *Hyphomicrobium*. Cells are about 0.7 μm wide. (b) Electron micrograph of a thin section of a single *Hyphomicrobium* cell. The hypha is about 0.2 μm wide.

or other microorganisms in aquatic habitats. Although a major function of these appendages is attachment, they also significantly increase the surface-to-volume ratio of the cells (prosthecae have large surface areas but almost no volume). Recall that the high surface-to-volume ratio of *prokaryotic* cells in general confers an increased ability to take up nutrients and expel wastes. The unusual morphology of appendaged bacteria (Figure 32) carries this theme to an extreme, and may be an evolutionary adaptation to life in *oligotrophic* (nutrient-poor) waters where these organisms are most commonly found. Prosthecae may also function to reduce cell sinking. Because these organisms are typically *strict aerobes*, prosthecae may keep cells from sinking into *anoxic* zones in their aquatic environments where they would be unable to respire.

Caulobacter and Gallionella

Two common stalked bacteria are *Caulobacter* (Figure 36) and *Gallionella* (Figure 38). The former is a *chemoorganotroph* that produces a cytoplasm-filled stalk, that is, a prostheca, while the latter is a *chemolithotrophic* iron-oxidizing bacterium whose stalk is composed of ferric hydroxide $[Fe(OH)_3]$. *Caulobacter* cells are often seen on surfaces in aquatic environments with the stalks of several cells attached to form rosettes (Figure 17.39a). At the end of the stalk is a structure called a holdfast by which the stalk anchors the cell to a surface.

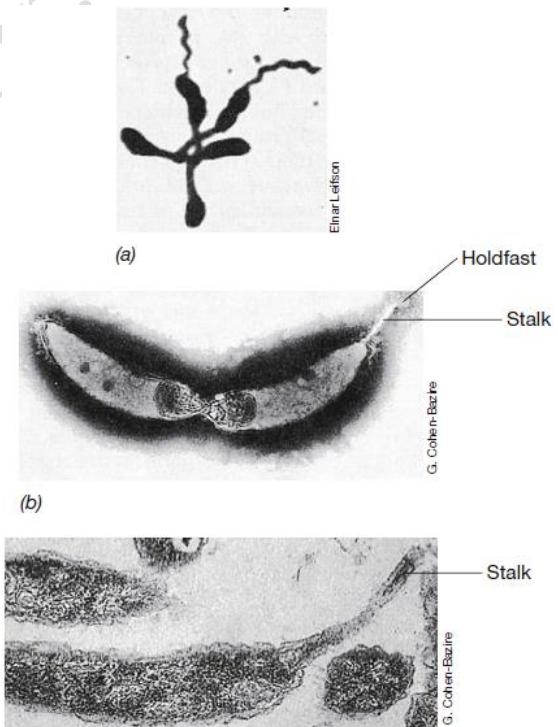


Figure 36: Stalked bacteria. (a) A *Caulobacter* rosette. A single cell is about $0.5 \mu m$ wide. The five cells are attached by their stalks, which are also prosthecae. Two of the cells have divided, and the daughter cells have formed flagella. (b) Negatively stained preparation of a *Caulobacter* cell in division. (c) A thin section of *Caulobacter* showing that cytoplasm is present in the stalk. Parts b and c are electron micrographs.

The *Caulobacter* cell division cycle (**Figure 37**) is unique because cells undergo unequal binary fission. A stalked cell of *Caulobacter* divides by elongation of the cell followed by binary fission, and a single flagellum forms at the pole opposite the stalk. The flagellated cell so formed, called a swarmer, separates from the

nonflagellated mother cell and eventually attaches to a new surface, forming a new stalk at the flagellated pole; the flagellum is then lost. Stalk formation is a necessary precursor of cell division and is coordinated with DNA synthesis (Figure 37). The cell division cycle in *Caulobacter* is thus more complex than simple binary fission or budding division because the stalked and swarmer cells are structurally different and the growth cycle must include both forms.

Gallionella forms a twisted stalk like structure containing Fe(OH)_3 from the oxidation of ferrous iron (Fe^{2+}) (**Figure 38**). However, the stalk of *Gallionella* is not an integral part of the cell but is simply excreted from the cell surface. It contains an organic matrix on which the Fe(OH)_3 accumulates. *Gallionella* is common in the waters draining bogs, iron springs, and other habitats where ferrous iron (Fe^{2+}) is present, usually in association with sheathed bacteria such as

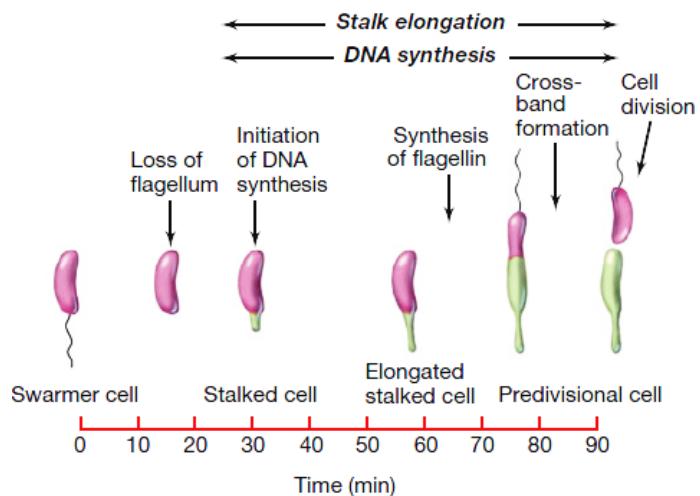


Figure 37: Growth of *Caulobacter*. Stages in the *Caulobacter* cell cycle, beginning with a swarmer cell.



Figure 38: The neutrophilic ferrous iron oxidizer, *Gallionella ferruginea*, from an iron seep near Ithaca, New York. (a) Photomicrograph of two bean-shaped cells with stalks that combine to form one twisted mass. (b) Transmission electron micrograph of a thin section of a *Gallionella* cell with stalk. Cells are about $0.6 \mu\text{m}$ wide.

Sphaerotilus. *Gallionella* is an autotrophic chemolithotroph containing enzymes of the Calvin cycle by which CO₂ is incorporated into cell material with Fe²⁺ as electron donor.

IV-Delta- and Epsilonproteobacteria

We round out the *Proteobacteria* with a consideration of the *Delta-* and *Epsilonproteobacteria*. As is typical of *Proteobacteria* in general, an assortment of metabolic patterns exists in species of these two *proteobacterial* classes, and several other phenotypic properties are unique to each group. We begin with the *myxobacteria*, socially active *prokaryotes* that interact to form macroscopically visible masses of cells.

1.17 Myxobacteria

Key Genera: *Myxococcus*, *Stigmatella*

Some bacteria exhibit a form of motility called *gliding*. Gliding bacteria, typically either long rods or filaments in morphology, lack flagella but can move when in contact with surfaces. One group of gliding bacteria, the *myxobacteria*, form multicellular structures called *fruiting bodies* and show life cycles involving intercellular communication. The fruiting *myxobacteria* are classified on morphological grounds using characteristics of the vegetative cells, the myxospores, and fruiting

Table 15 Classification of the fruiting myxobacteria^a

Characteristics	Genus
Vegetative cells tapered	
Spherical or oval myxospores, fruiting bodies usually soft and slimy without well-defined sporangia or stalks	<i>Myxococcus</i>
Rod-shaped myxospores:	<i>Archangium</i>
Myxospores not contained in sporangia, fruiting bodies without stalks	
Myxospores embedded in slime envelope:	
Fruiting bodies without stalks	<i>Cystobacter</i>
Stalked fruiting bodies, single sporangia	<i>Melittangium</i>
Stalked fruiting bodies, multiple sporangia	<i>Stigmatella</i>
Fruiting bodies are dark-brown clusters consisting of tiny spherical or disclike sporangia with an outer wall	<i>Angiococcus</i>
Vegetative cells not tapered (blunt, rounded ends); myxospores resemble vegetative cells; sporangia always produced	
Fruiting bodies without stalks; myxospores rod-shaped	<i>Polyangium</i>
Fruiting bodies without stalks; myxospores oval; highly cellulolytic	<i>Sorangium</i>
Fruiting bodies without stalks; myxospores coccoid	<i>Nannocystis</i>
Stalked fruiting bodies	<i>Chondromyces</i>

^aPhylogenetically, all known myxobacteria are *Delta*proteobacteria.

body structure (**Table 15**), and on phylogenetic grounds, using 16S rRNA gene sequence analyses to differentiate these different species of *Delta**proteobacteria*.

Fruiting Bodies

The fruiting *myxobacteria* exhibit the most complex behavioral patterns and life cycles of all known bacteria. To encode this complexity, the chromosome of some *myxobacteria* is very large. *Myxococcus xanthus*, for example, has a single circular chromosome of 9.2 megabase pairs, twice the size of the *Escherichia coli* chromosome. The vegetative cells of the fruiting *myxobacteria* are simple, nonflagellated, gram-negative rods that glide across surfaces and obtain their nutrients primarily by lysing other bacteria and utilizing the released nutrients. Under appropriate conditions, a swarm of vegetative cells aggregate and form fruiting bodies, within which some of the cells become converted to resting structures called myxospores.

The fruiting bodies of the *myxobacteria* vary from simply masses of myxospores embedded in slime to complex forms with a fruiting body wall and a stalk (**Figure 39**). The fruiting bodies are often strikingly colored and morphologically elaborate (**Figure 40**), and can often be seen with the aid of a hand lens forming on moist pieces of decaying wood or plant material. **Fruiting bodies** of *myxobacteria* often develop on dung pellets (for example, rabbit pellets)

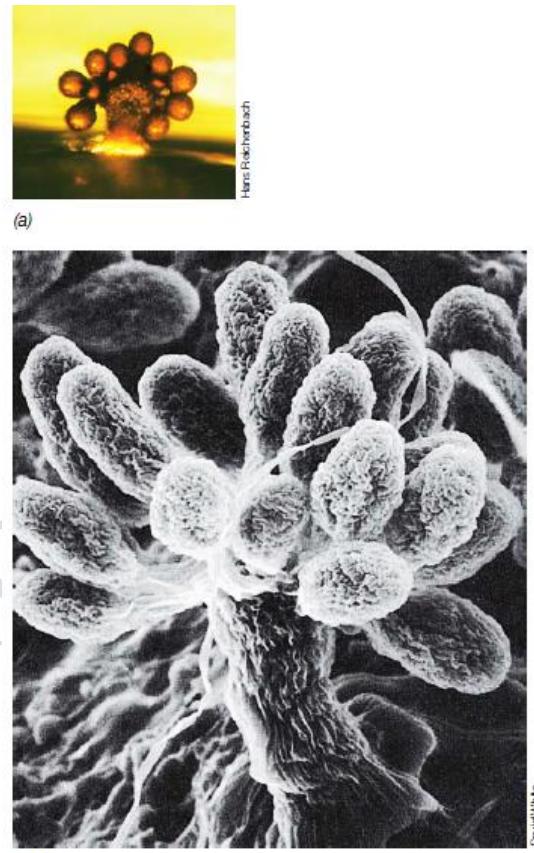


Figure 39: *Stigmatella aurantiaca*. (a) Color photo of a single fruiting body. The structure is about 150 μm high. (b) Scanning electron micrograph of a fruiting body growing on a piece of wood. Note the individual cells visible in each fruit. The color of the fruiting body shown in part a is due to the production of structurally complex glucosylated carotenoid pigments.

after the pellets have been incubated for a few days in a moist chamber, and by using an inoculating loop or needle, one can transfer cells from a fruiting body to a plate for isolation. Many *myxobacteria* can be grown in the laboratory on complex media containing peptone or casein hydrolysate, which provides amino acids or small peptides as nutrients, and most are *obligate aerobes*.

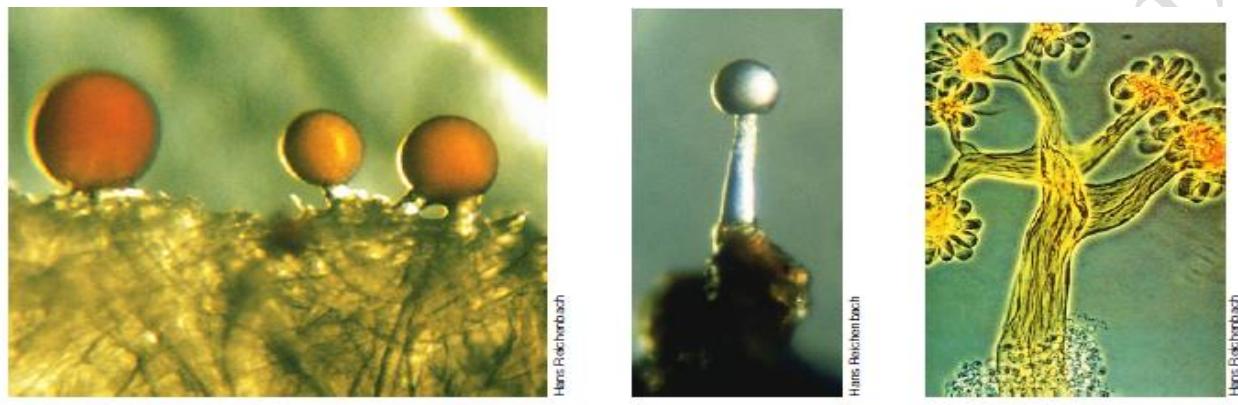


Figure 40: Fruiting bodies of three species of fruiting myxobacteria. (a) *Myxococcus fulvus* (125 µm high). (b) *Myxococcus stipitatus* (170 µm high). (c) *Chondromyces crocatus* (560 µm high).

Life Cycle of a Fruiting Myxobacterium

The life cycle of a typical fruiting *myxobacterium* is shown in **Figure 41**. A vegetative cell excretes slime, and as it moves across a solid surface, it leaves behind a slime trail (**Figure 42**). This slime trail is then used by other cells, such that a characteristic radiating pattern soon emerges with cells migrating along established slime trails (Figure 42). The fruiting body ultimately formed (Figures 39 and 40) is a complex structure produced by cells that synthesize a stalk and those in the head that form myxospores.

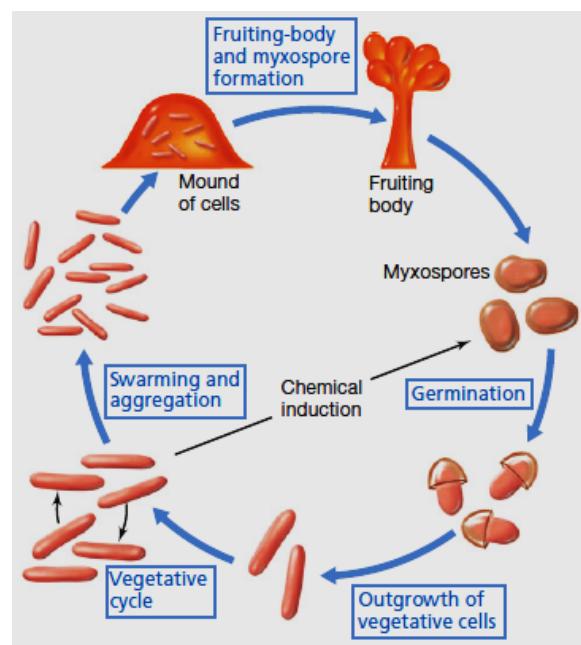


Figure 41: Life cycle of *Myxococcus xanthus*. Aggregation assembles vegetative cells that then undergo fruiting body formation, within which some vegetative cells undergo morphogenesis to form resting cells called myxospores. The myxospores germinate under favorable nutritional and physical conditions to yield vegetative cells.

Fruiting bodies do not form if adequate nutrients for vegetative growth are present, but upon nutrient exhaustion, the vegetative swarms begin to fruit. Cells aggregate, likely through chemotactic or quorum sensing responses, with the cells migrating toward each other and forming mounds or heaps (**Figure 43**); a single fruiting body may have more than a billion cells. As the cell masses become higher, the differentiation of the fruiting body into stalk and head begins.

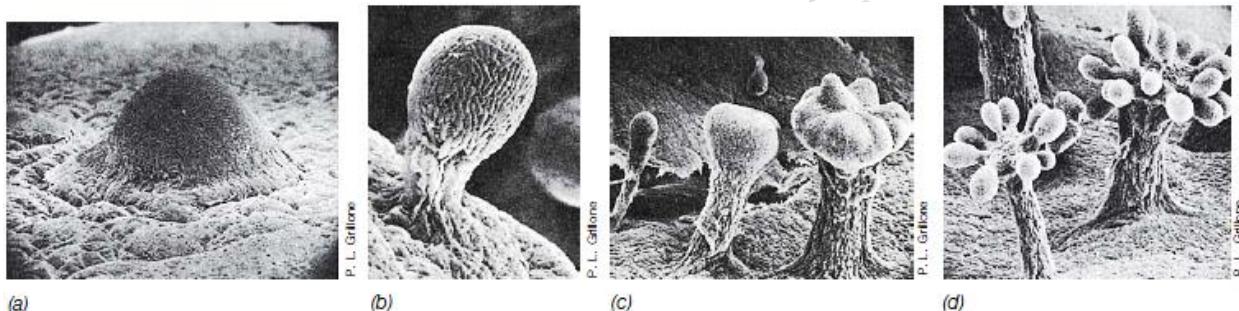


Figure 43: Scanning electron micrographs of fruiting body formation in *Chondromyces crocatus*. (a) Early stage, showing aggregation and mound formation. (b) Initial stage of stalk formation. Slime formation in the head has not yet begun and so the cells that compose the head are still visible. (c) Three stages in head formation. Note that the diameter of the stalk also increases. (d) Mature fruiting bodies. The entire fruiting structure is about 600 μm in height.

The stalk is composed of slime within which a few cells are trapped. The majority of the cells migrate to the fruiting body head, where they undergo differentiation into myxospores (Figures 39–43). Compared to vegetative cells, myxospores are more resistant to drying, ultraviolet radiation, and heat, but the degree of heat resistance is much less than that of the bacterial endospore. The main function of the myxospore is probably to allow the organism to survive desiccation during dispersal or during intermittent drying of the habitat. Upon dissemination to a suitable habitat or restoration of adequate growth conditions, the myxospore eventually germinates to form a new vegetative cell.

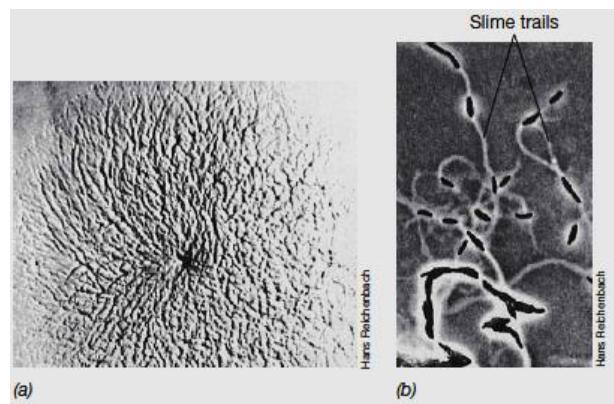


Figure 42: Swarming in *Myxococcus*. (a) Photomicrograph of a swarming colony (5-mm radius) of *Myxococcus xanthus* on agar. (b) Single cells of *Myxococcus fulvus* from an actively gliding culture, showing the characteristic slime trails on the agar. A cell of *M. fulvus* is about 0.8 μm in diameter.

Many myxobacteria synthesize carotenoid pigments and thus their fruiting bodies are brightly colored (Figures 39a and 40). Pigment formation is promoted by light, and at least one function of these pigments is likely photoprotection, which would be beneficial in nature since the myxobacteria are typically exposed to sunlight. In the genus *Stigmatella* (Figure 39), light greatly stimulates fruiting body formation and catalyzes production of the compound 2,5,8-trimethyl-8-hydroxy-nonane-4-one. This substance, a type of *pheromone*, promotes cell aggregation, the initial step in fruiting body formation (Figure 41).

1.18 Sulfate- and Sulfur-Reducing *Proteobacteria*

Key Genera: *Desulfovibrio*, *Desulfobacter*, *Desulfomonas*

Sulfate (SO_4^{2-}) and sulfur (S^0) are electron acceptors for a large group of *anaerobic Deltaproteobacteria* that utilize organic compounds or H_2 as electron donors in *anaerobic* respirations. Hydrogen sulfide (H_2S) is the product of both SO_4^{2-} and S^0 reduction. Over 40 genera of these organisms, collectively called the dissimilative **sulfate-reducing bacteria** and **sulfur-reducing bacteria**, are known, and some of the key ones are shown in **Table 16**. The word dissimilative refers to the use of SO_4^{2-} or S^0 as electron acceptors in energy generation instead of their assimilation as biosynthetic sources of sulfur.

General Properties

The genera of dissimilative sulfate-reducing bacteria form two physiological groups, those that can oxidize acetate and other fatty acids completely to CO_2 , and those that cannot. The latter group includes the best studied of sulfate-reducing bacteria, *Desulfovibrio*, along with *Desulfomonas*, *Desulfotomaculum*, and *Desulfobulbus* (**Figure 44**). These organisms utilize lactate, pyruvate, ethanol, or certain fatty acids as electron donors, reducing SO_4^{2-} to H_2S . The acetate oxidizers include *Desulfobacter* (Figure 44d), *Desulfococcus*, *Desulfosarcina* (Figure 44e),

and *Desulfonema* (Figure 44b), among many others, and specialize in the complete oxidation of fatty acids, in particular acetate, reducing SO_4^{2-} to H_2S . The sulfate-reducing bacteria are, for the most part, *obligate anaerobes*, and *strict anoxic* techniques must be used in their cultivation (Figure 44g).

Table 16 Characteristics of some key genera of sulfate- and sulfur-reducing bacteria^a

Genus	Characteristics
Species unable to oxidize acetate	
<i>Desulfovibrio</i>	Polarly flagellated, curved rods, gram-negative; contain desulfovibrin; one thermophilic
<i>Desulfomicrobium</i>	Motile rods, gram-negative; desulfovibrin absent
<i>Desulfobulbus</i>	Vibrios; gram-negative; motile; desulfovibrin absent
<i>Desulfovustis</i>	Motile rods, specialize in the degradation of glycolate and glyoxalate
<i>Desulfotomaculum</i>	Straight or curved rods; motile by peritrichous or polar flagellation; gram-negative; desulfovibrin absent; produce endospores; capable of utilizing acetate as energy source; related to <i>Firmicutes</i>
<i>Desulfomonile</i>	Rods; capable of reductive dechlorination of 3-chlorobenzoate to benzoate (Section 14.12)
<i>Desulfobacula</i>	Oval to coccoid cells, marine; can oxidize various aromatic compounds including the aromatic hydrocarbon toluene to CO_2
<i>Archaeoglobus</i>	Archaeon; hyperthermophile, temperature optimum 83°C; contains some unique coenzymes of methanogenic bacteria, makes small amount of methane during growth; H_2 , formate, glucose, lactate, and pyruvate are electron donors; SO_4^{2-} , $\text{S}_2\text{O}_3^{2-}$, or SO_3^{2-} are electron acceptors (Section 19.6)
<i>Desulfobulbus</i>	Ovoid or lemon-shaped cells, gram-negative; desulfovibrin absent; if motile, by single polar flagellum; utilizes propionate as electron donor with acetate + CO_2 as products
<i>Desulforhopalus</i>	Curved rods, gas vacuolate, psychrophile; uses propionate, lactate, or alcohols as electron donor
<i>Thermodesulfobacterium</i>	Small, gram-negative rods; desulfovibrin present; thermophilic, optimum growth at 70°C; a member of the <i>Bacteria</i> but contains ether-linked lipids (Section 18.19)
Acetate-oxidizing species	
<i>Desulfobacter</i>	Rods, gram-negative; desulfovibrin absent; if motile, by single polar flagellum; utilizes only acetate as electron donor and oxidizes it to CO_2 via the citric acid cycle
<i>Desulfobacterium</i>	Rods, some with gas vesicles, marine; capable of autotrophic growth via the acetyl-CoA pathway
<i>Desulfococcus</i>	Spherical cells; nonmotile; gram-negative; desulfovibrin present; utilizes C ₁ to C ₁₄ fatty acids as electron donor with complete oxidation to CO_2 ; capable of autotrophic growth via the acetyl-CoA pathway
<i>Desulfonema</i>	Large, filamentous gliding bacteria, gram-positive; desulfovibrin present or absent; utilizes C ₂ to C ₁₂ fatty acids as electron donor with complete oxidation to CO_2 ; capable of autotrophic growth via the acetyl-CoA pathway (H_2 as electron donor)
<i>Desulfosarcina</i>	Cells in packets (sarcina arrangement), gram-negative; desulfovibrin absent; utilizes C ₂ to C ₁₄ fatty acids as electron donor with complete oxidation to CO_2 ; capable of autotrophic growth via the acetyl-CoA pathway (H_2 as electron donor)
<i>Desulfarculus</i>	Vibrios; gram-negative; motile; desulfovibrin absent; utilizes only C ₁ to C ₁₈ fatty acids as electron donor
<i>Desulfacinum</i>	Cocci to oval-shaped cells; gram-negative; utilizes C ₁ to C ₁₈ fatty acids, very nutritionally diverse, capable of autotrophic growth; thermophilic
<i>Desulforhabdus</i>	Rods, gram-negative; nonmotile; utilizes fatty acids with complete oxidation to CO_2
<i>Thermodesulforhabdus</i>	Gram-negative motile rods; thermophilic; uses fatty acids up to C ₁₈
Dissimilative sulfur reducers	
<i>Desulfomonas</i>	Straight rods, single lateral flagellum, gram-negative; does not reduce sulfate; acetate, succinate, ethanol, or propanol used as electron donor; obligate anaerobe; one species is capable of the reductive dechlorination of trichloroethylene (Section 14.12)
<i>Desulfurella</i>	Motile short rods; gram-negative; requires acetate; thermophilic
<i>Sulfurospirillum</i>	Small vibrios, reduces S ⁰ with H_2 or formate as electron donors
<i>Campylobacter</i>	Curved, vibrio-shaped rods with polar flagella, gram-negative; unable to reduce sulfate but can reduce sulfur, sulfite, thiosulfate, nitrate, or fumarate anaerobically with acetate or a variety of other carbon or electron donor sources; facultative aerobe; microaerophilic

Sulfate-reducing bacteria are widespread in aquatic and terrestrial environments that contain some SO_4^{2-} and become *anoxic* as a result of microbial decomposition processes. *Desulfotomaculum*, phylogenetically a member of the *Firmicutes* (gram-positive Bacteria), consists of endospore-forming rods found primarily in soil. Growth and reduction of SO_4^{2-} by *Desulfotomaculum* in certain canned foods leads to a type of spoilage called **sulfide stinker**. The remaining genera of sulfate reducers are indigenous to *anoxic* freshwater or marine environments and can occasionally be isolated from the mammalian intestine.

Dissimilative Sulfur Reduction

The dissimilative sulfur-reducing bacteria can reduce S^0 to H_2S but are unable to reduce SO_4^{2-} . Species of *Desulfuromonas* (Figure 44f) grow *anaerobically* by coupling the oxidation of acetate or a few other organic compounds to the reduction of S^0 .

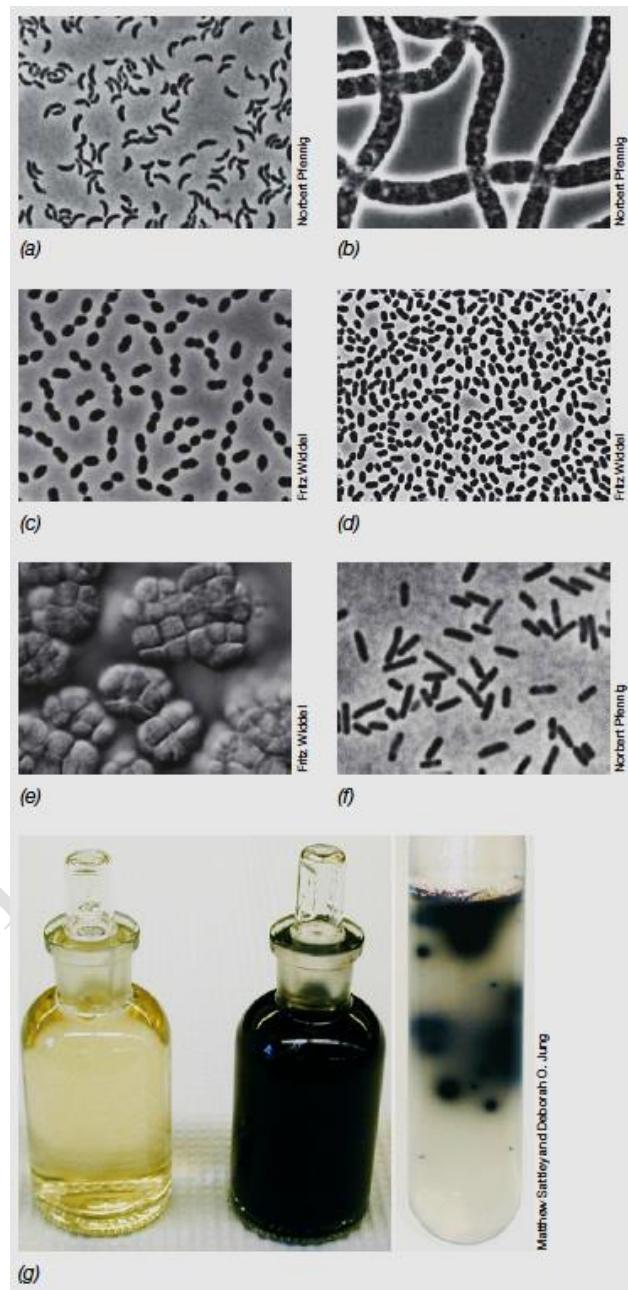


Figure 44: Representative sulfate-reducing and sulfur-reducing bacteria. (a) *Desulfovibrio desulfuricans*; cell diameter about 0.7 μm . (b) *Desulfonema limicola*; cell diameter 3 μm . (c) *Desulfovibulus propionicus*; cell diameter about 1.2 μm . (d) *Desulfobacter postgatei*; cell diameter about 1.5 μm . (e) *Desulfosarcina variabilis*; cell diameter about 1.25 μm . (f) *Desulfuromonas acetoxidans*; cell diameter about 0.6 μm . (g) Enrichment culture of sulfate-reducing bacteria. Left, sterile medium; center, a positive enrichment showing black FeS ; right, colonies of sulfate-reducing bacteria in a dilution tube. Photos a–d and f are phase-contrast photomicrographs; part e is an interference contrast micrograph.

However, the ability to reduce S^0 , as well as other sulfur compounds such as thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}), or dimethyl sulfoxide (DMSO), is widespread in a number of *chemoorganotrophic*, generally *facultatively aerobic* bacteria (for example, *Proteus*, *Campylobacter*, *Pseudomonas*, and *Salmonella*). *Desulfuromonas* differs from these *aerobic* bacteria in that it is an *obligate anaerobe* and utilizes only S^0 as an electron acceptor (Table 16). Dissimilative sulfur-reducing bacteria reside in many of the same habitats as dissimilative sulfate-reducing bacteria and often form associations with bacteria that oxidize H_2S to S^0 , such as green sulfur bacteria. The S^0 produced from H_2S oxidation is then reduced back to H_2S during metabolism of the sulfur reducer, completing an *anoxic* sulfur cycle.

Physiology of Sulfate-Reducing Bacteria

Here we consider some of the more general physiological properties of this group. The range of electron donors used by sulfate-reducing bacteria is fairly broad. Hydrogen (H_2), lactate, and pyruvate are almost universally used and many species also oxidize certain alcohols (for example, ethanol, propanol, and butanol) as electron donors. Some strains of *Desulfotomaculum* utilize glucose, but this is rare among sulfate reducers.

Desulfovibrio species typically oxidize lactate, pyruvate, or ethanol to acetate and then excrete this fatty acid as an end product. Depending on the species, fatty acid-oxidizing, sulfate reducing bacteria oxidize acetate as well as longer chain fatty acids (Table 16) completely to CO_2 . Some species, such as *Desulfosarcina* and *Desulfonema*, grow *chemolithotrophically* and *autotrophically* with H_2 as an electron donor, SO_4^{2-} as an electron acceptor, and CO_2 as the sole carbon source. A few sulfate reducers can oxidize individual hydrocarbons and even crude oil itself as electron donors. A few species of sulfate reducing bacteria also fix nitrogen, but this property is not common among sulfate-reducing bacteria.

In addition to using SO_4^{2-} as an electron acceptor, many sulfate-reducing bacteria can use nitrate; sulfonates, such as isethionate ($\text{HO}-\text{CH}_2-\text{CH}_2-\text{SO}_3^-$); and S^0 .

Certain organic compounds can also be fermented by sulfate reducing bacteria. The most common of these is pyruvate, which is fermented by way of the phosphoroclastic reaction to acetate, CO_2 , and H_2 . Moreover, although generally *obligate anaerobes*, a few sulfate-reducing bacteria, primarily strains isolated from microbial mats where they coexist with O_2^- - producing *cyanobacteria*, are quite O_2^- -tolerant and can respire with O_2 as the electron acceptor. At least one species, *Desulfovibrio oxydilnace*, can actually grow with O_2 as the electron acceptor under *microaerophilic* conditions.

Isolation

The enrichment of *Desulfovibrio* species is easy in an *anoxic* lactate– sulfate medium containing ferrous iron (Fe^{2+}). A reducing agent, such as thioglycolate or ascorbate, is required to achieve a low reduction potential (E_0') in the medium. When sulfate-reducing bacteria grow, the H_2S formed from SO_4^{2-} reduction combines with the ferrous iron to form black, insoluble ferrous sulfide (Figure 44g). This blackening not only indicates sulfate reduction, but the iron also binds and detoxifies the H_2S , making possible growth to higher cell densities. Purification can be accomplished by diluting the culture in molten agar tubes; a small amount of liquid from the original enrichment is added to a tube of molten agar growth medium, mixed thoroughly, and sequentially diluted through a series of molten agar tubes. Upon solidification, individual cells of sulfate-reducing bacteria become distributed throughout the agar and grow to form black colonies (Figure 44g) that can be removed aseptically to yield pure cultures.

1.19 The *Epsilonproteobacteria*

The *Epsilon* class of *Proteobacteria* was initially defined by only a few pathogenic bacteria; in particular, by species of *Campylobacter* and *Helicobacter*. However, environmental studies of marine and terrestrial microbial habitats have shown that a diversity of *Epsilonproteobacteria* exist in nature, and their numbers and metabolic capabilities suggest they play important ecological roles. Species of *Epsilonproteobacteria* are especially abundant at *oxic–anoxic* interfaces in sulfur-rich environments, such as those surrounding hydrothermal vents; in these habitats they catalyze metabolic transformations of sulfur and associate with animals that live near the vents. Many of these bacteria are *autotrophs* and use H₂, formate, or reduced sulfur compounds, with nitrate, oxygen, or elemental sulfur as electron acceptors, depending on the species. Here we describe major groups of cultured *Epsilonproteobacteria* and briefly consider the diversity of uncultured members of this class.

Campylobacter and *Helicobacter*

These two genera are key representatives of the *Epsilonproteobacteria* that share a number of characteristics. They are all gram-negative, motile spirilla, and most species are pathogenic to humans or other animals (**Table 17**). These organisms are also *microaerophilic* and must therefore be cultured from clinical specimens in culture media incubated at low (3–15%) O₂ and high (3–10%) CO₂. *Campylobacter* species, over a dozen of which have been described, cause acute gastroenteritis that typically results in a bloody diarrhea. Pathogenesis is due to several factors, including an enterotoxin that is related to cholera toxin. *Helicobacter pylori*, also a pathogen, causes both chronic and acute gastritis, leading to the formation of peptic ulcers. We consider these diseases, including their modes of transmission and clinical symptoms.

Table 17 Characteristics of key genera of Epsilonproteobacteria

Genus	Habitat	Descriptive characters	Physiology and metabolism
<i>Campylobacter</i>	Reproductive organs, oral cavity, and intestinal tract of humans and other animals; pathogenic	Slender, spirally curved rods; corkscrew-like motility by single polar flagellum	Microaerophilic; chemoorganotrophic
<i>Arcobacter</i>	Diverse habitats (freshwater, sewage, saline environments, animal reproductive tract, plants); some species pathogenic for humans and other animals	Slender, curved rods; motile by single polar flagellum	Microaerophilic; aerotolerant or aerobic; chemoorganotrophic; oxidation of sulfide to elemental sulfur (S^0) by some species; nitrogen fixation in one species
<i>Helicobacter</i>	Intestinal tract and oral cavity of humans and other animals; pathogenic	Rods to tightly spiral; some species with tightly coiled periplasmic fibers	Microaerophilic, chemoorganotrophic; produce high levels of urease (nitrogen assimilation)
<i>Sulfurospirillum</i>	Freshwater and marine habitats containing sulfur	Vibrioid to spiral-shaped cells; motile by polar flagella	Microaerophilic; reduces elemental sulfur (S^0)
<i>Thiovulum</i>	Freshwater and marine habitats containing sulfur; not yet in pure culture	Cells contain orthorhombic S^0 granules; rapid motility by peritrichous flagella	Microaerophilic; chemolithotrophic oxidizing H_2S
<i>Wolinella</i>	Bovine rumen	Rapidly motile by polar flagellum; single species known: <i>W. succinogenes</i>	Anaerobe; anaerobic respiration using fumarate, nitrate, or other compounds as terminal electron acceptor, and with H_2 or formate as electron donor

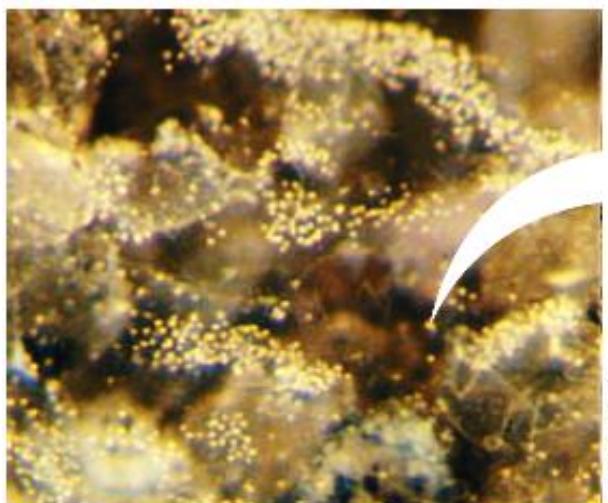
Arcobacter

The genus *Arcobacter*, a relative of *Campylobacter*, is unusual among *Epsilonproteobacteria* in that its various species show an unusually wide diversity of habitats. Some species are pathogenic, infecting the reproductive and intestinal tracts of humans and other animals where they can cause reproductive failures, diarrhea-like diseases, and *gastroenteritis* and *appendicitis*. *Arcobacter* species have also been found in sewage and water reservoirs, so a fecal–oral route is likely the means by which gastrointestinal infections of *Arcobacter* are transmitted. One species, *Arcobacter nitrofigilis*, is associated with sediment and the roots of the salt marsh plant *Spartina* and can fix nitrogen.

Sulfurospirillum, *Thiovulum*, and *Wolinella*

Species of *Sulfurospirillum*, a *Campylobacter* relative, are nonpathogenic, free-living *microaerophiles* found in freshwater and marine habitats. These bacteria also carry out *anaerobic* respirations using elemental sulfur (S^0), selenate, or arsenate as electron acceptors.

Thiovulum also is *microaerophilic* and is found in freshwater and marine habitats in which sulfiderich muds interface with oxic zones. At such interfaces populations of *Thiovulum* oxidize H₂S formed by sulfate-reducing bacteria and store the product, S⁰, as globules inside the cell (**Figure 45**). When motile, *Thiovulum* cells, which are *peritrichously flagellated*, swim at exceptionally high speed, perhaps the fastest of all known bacteria (~0.6 mm/sec). *Thiovulum* cells are fairly large (10–20 µm) and secrete a slime that forms a veil-like film that helps cells attach to solid surfaces such as sand grains (Figure 45a). *Thiovulum* is presumably a *chemolithotrophic autotroph* but pure cultures of the organism have yet to be achieved to rigorously test this hypothesis. However, if it is true, the physiology and ecology of *Thiovulum* would be very similar to other colorless sulfur bacteria such as *Achromatium*, *Beggiatoa*, and *Thiobacillus*, sulfur *chemolithotrophs* that reside in other classes of *Proteobacteria* (Figure 2).



Tom Fenchel



Tom Fenchel

(a)

(b)

Figure 45: The sulfur-oxidizing *epsilonproteobacterium* *Thiovulum*. (a) Macrograph of cells of *Thiovulum* (yellow dots) that formed a thin veil in marine sand containing H₂S (large, irregular structures are sand grains). (b) Transmission electron micrograph of a dividing cell of *Thiovulum*. Sulfur (S⁰) globules are shown with arrows. Single cells of *Thiovulum* are typically 10–20 µm in diameter.

Wolinella is an *anaerobic* bacterium isolated from the bovine rumen (Table 17). Unlike other *Epsilonproteobacteria*, the single known species, *W.*

succinogenes, grows best as an *anaerobe* and can catalyze *anaerobic* respirations using fumarate or nitrate as electron acceptors with H₂ or formate as electron donors. Although *W. succinogenes* has thus far been found only in the rumen, its genome shows significant homologies to both the *Campylobacter* and *Helicobacter* genomes and contains additional genes that encode nitrogen fixation, extensive cell signaling mechanisms, and virtually complete metabolic pathways, absent from closely related genomes. This suggests that *Wolinella* inhabits diverse environments outside of the rumen.

Environmental *Epsilonproteobacteria*

In addition to cultured representatives of the genera mentioned above, and many additional species and genera not considered here, there are large groups within this class that are known only from 16S ribosomal RNA gene sequences obtained from the environment. Through environmental sequencing studies and ongoing cultivation efforts, species of *Epsilonproteobacteria* are now becoming recognized as ubiquitous in marine and terrestrial environments where sulfur-cycling activities are ongoing, particularly in deep-sea hydrothermal vent habitats where sulfide-rich and oxygenated waters mix. Also, living attached to the surface of animals such as the tube worm *Alvinella* and the shrimp *Rimicaris* that reside near hydrothermal vents, a large variety of as yet uncultured *Epsilonproteobacteria* may, through their sulfur metabolism, detoxify H₂S that would otherwise be deleterious to their animal hosts, allowing the animals to thrive in a chemically hostile environment. It is thus likely that further exploration of the phylogeny, metabolic activities, and ecological roles of *Epsilonproteobacteria* will generate exciting new aspects of *prokaryotic* diversity.

Key Terms & Glossary:

Autoinduction a gene regulatory mechanism involving small, diffusible signal molecules that are produced in larger amounts as population size increases.

Bioluminescence the enzymatic production of visible light by living organisms.

Carboxysome a polyhedral cellular inclusion of crystalline ribulose bisphosphate carboxylase (RubisCO), the key enzyme of the Calvin cycle.

Chemolithotroph an organism able to oxidize inorganic compounds (such as H₂, Fe²⁺, S⁰, or NH₄⁺) as energy sources (electron donors).

Enteric bacteria a large group of gram negative *rod*-shaped Bacteria characterized by a *facultatively aerobic* metabolism and commonly found in the intestines of animals.

Methanotroph an organism capable of oxidizing methane (CH₄) as an electron donor in energy metabolism.

Methylotroph an organism capable of oxidizing organic compounds that do not contain carbon–carbon bonds; if able to oxidize CH₄, also a methanotroph.

Mixotroph an organism that can conserve energy from the oxidation of inorganic compounds but requires organic compounds as a carbon source.

Nitrifying bacteria *chemolithotrophs* capable of carrying out the transformation NH₃ → NO₂⁻, or NO₂⁻ → NO₃⁻

Prosthecae extrusions of cytoplasm, often forming distinct appendages, bounded by the cell wall.

Proteobacteria a major lineage of Bacteria that contains a large number of gram-negative *rods* and *cocci*.

Purple nonsulfur bacteria a group of phototrophic bacteria containing bacteriochlorophyll a or b and that grow best as *photoheterotrophs* and without H₂S.

Purple sulfur bacteria a group of *phototrophic* bacteria containing bacteriochlorophylls a or b and that can oxidize H₂S and store elemental sulfur inside the cells (or in some species, outside the cell).

Spirilla spiral-shaped cells (singular, *spirillum*).

Sulfate-reducing and sulfur-reducing bacteria two groups of Bacteria that respire *anaerobically* with SO₄²⁻ or S⁰, respectively, as electron acceptors, producing H₂S as final product.

Proteobacteria are a huge class of gram-negative bacteria characterized by a wide distribution in nature and broad metabolic diversity. Many bacteria commonly cultured from soil, water, and animal bodies are species of *Proteobacteria*.

Phototrophic purple bacteria are *anoxygenic* phototrophs that grow *phototrophically* and obtain carbon from CO₂ plus H₂S (purple sulfur bacteria) or organic compounds (purple nonsulfur bacteria). Purple nonsulfur bacteria are physiologically diverse, and most can also grow as *chemoorganotrophs* in darkness. Purple bacteria belong to the *Alpha-, Beta-, and Gammaproteobacteria*.

The nitrifying bacteria include *chemolithotrophs* that can oxidize NH₃ to NO₂⁻ (*Nitrosomonas*) and NO₂⁻ to NO₃⁻ (*Nitrobacter*). These organisms grow *autotrophically* in *oxic* nitrogen-rich habitats.

Sulfur chemolithotrophs oxidize H₂S and other reduced sulfur compounds for energy metabolism with O₂ or NO₃⁻ as electron acceptors and use either CO₂ or organic compounds as carbon sources.

The hydrogen bacteria oxidize H₂ with O₂ as the electron acceptor and fix CO₂ as the carbon source. Some hydrogen bacteria, the carboxydobacteria, oxidize

carbon monoxide (CO). Most of these bacteria can also grow on organic compounds.

Methylotrophs are bacteria able to grow on carbon compounds that lack carbon–carbon bonds. Some *methylotrophs* are also *methanotrophs*, organisms able to catabolize methane. Most *methanotrophs* are *Proteobacteria* that contain extensive internal membranes and incorporate C₁ units by way of either the serine or ribulose monophosphate pathway. *Methanotrophs* reside in water and soil and are also symbionts of certain marine animals.

Pseudomonads include many gram-negative, *chemoorganotrophic*, *aerobic* rods, some of which are pathogenic. The genus *Pseudomonas* includes many species that are nutritionally diverse and widespread in nature in soil, water, and on the surfaces of plants and animals.

The acetic acid bacteria *Acetobacter* and *Gluconobacter* produce acetate from the oxidation of ethanol, and these acid-tolerant bacteria are often found in the fermenting fluids of alcoholic beverages.

The free-living nitrogen-fixing bacteria are *obligately aerobic* bacteria that can use N₂ as their nitrogen source. A common genus is *Azotobacter*, an organism that produces slimy colonies in laboratory cultures.

Neisseria, *Chromobacterium*, and their relatives can be isolated from animals, and some species of this group are pathogenic, causing severe diseases in humans including *gonorrhea* and one form of *meningitis*.

Enteric bacteria are a major group of highly related, gram-negative, *facultative* bacteria of major medical importance. The different genera are distinguished primarily on phenotypic grounds, including metabolic properties, such as fermentation patterns.

Vibrio, *Aliivibrio*, and *Photobacterium* species are marine organisms, some of which are pathogenic and *bioluminescent*. *Bioluminescence*, catalyzed by the

enzyme *luciferase*, is controlled by a quorum-sensing mechanism that ensures that light is not emitted until a large cell population has been attained.

Rickettsias are obligate intracellular parasites that are deficient in many metabolic functions and obtain key metabolites from their hosts. Species of the genus *Rickettsia* cause diseases such as typhus and Rocky Mountain spotted fever, while the genus **Wolbachia** contains common insect endosymbionts.

Spirilla are spiral-shaped, *chemoorganotrophic* bacteria that are widespread in the aquatic environment. Species are distributed among five classes of *Proteobacteria*. *Magnetospirillum* displays **magnetotaxis** and *Bdellovibrio* is a predatory bacterium, attacking and killing other gram-negative bacteria.

Sheathed bacteria are filamentous *Proteobacteria* in which individual cells form chains within an outer layer called the **sheath**. *Sphaerotilus* and *Leptothrix* are major genera of sheathed bacteria and can oxidize metals, such as Fe^{2+} and Mn^{2+} .

Budding and prosthecate bacteria are appendaged cells that form stalks or prosthecae used for attachment or nutrient absorption and are primarily aquatic. *Hypomicrobium*, *Caulobacter*, and *Gallionella* are major genera.

The fruiting myxobacteria are rod-shaped gliding bacteria that aggregate to form complex masses of cells called **fruiting bodies**. *Myxobacteria* are mostly *aerobic chemoorganotrophic* soil bacteria that live by consuming dead organic matter or other bacterial cells.

Sulfate- and sulfur-reducing bacteria are a large group of *Delta proteobacteria* unified physiologically by their ability to reduce SO_4^{2-} or S^0 to H_2S under *anoxic* conditions. Two physiological classes of sulfate-reducing bacteria are known, those that are capable of oxidizing acetate to CO_2 and those that are not.

Epsilonproteobacteria contain both pathogenic species, such as *Campylobacter* and *Helicobacter*, and nonpathogenic species, such as *Sulfospirillum* and *Thiovulum*. Many of the latter inhabit the *oxic-anoxic* interface of sulfide-rich environments.

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