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# MICROBIAL DIVERSITY IN THE ENVIRONMENT

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## 2.1 INTRODUCTION

The microbial world in the environment is highly diverse with a lot of variation in their morphology, physiology and metabolism. The metabolic diversity and genetic elasticity of the microbial groups has enabled them to show their presence in each and every corner of the earth, ranging from a favourable mesophytic habitat to an extremely unfavourable deep sea vent. In environmental microbiology, we categorize microbes into two distinct domains: as prokaryotes and eukaryotes, both of which are intricately involved in all environmental processes. The smallest organisms are the bacteria with a diameter less than a micrometer to several micrometers. The eukaryotic microorganisms are bigger in size and have more structural complexity. However, a detailed study of all the microorganisms is beyond the scope of this book. This chapter contains only an abstract of diversity of the microbial world. Many of these microbial groups will be dealt in detail in subsequent chapters.

## 2.2 CLASSIFICATION OF MICROBES

Because of the bewildering diversity of microorganisms, it is desirable to classify or arrange them into groups based on their mutual similarities. People often think of microbial taxonomy as trivial and boring, simply a matter of their arrangement on the basis of some physical or metabolic similarities, which may not be required always. Actually, classification of microbes is often important for several reasons. First, it allows us to organize huge amount of knowledge about organisms because all members of a particular group share many characteristics. The more accurate the classification, the more information-rich and useful it is, especially to culture and make use of the organisms. Second, classification of microbes, basing on their metabolic requirements, allows us to make predictions and frame hypotheses for further research based on knowledge of similar organisms. It also facilitates the application of the microorganisms for efficient degradation of

**Table 2.1:** The common morphological, physiological and metabolic features used in classification and identification of bacteria.

<i>Morphological features</i>	<i>Physiological features</i>	<i>Metabolic features</i>
Cell shape	Carbon and nitrogen sources	Energy sources
Cell size	Cell wall constituents	Fermentation products
Colonial morphology	General nutritional type	Luminescence
Ultrastructural characteristics	Growth temperature optimum and range	Mechanisms of energy conversion
Staining behaviour	Osmotic tolerance	Oxygen relationships
Cilia and flagella	pH optimum and growth range	Photosynthetic pigments
Mechanism of motility	Salt requirements and tolerance	Secondary metabolites formed
Endospore shape and location		Sensitivity to metabolic inhibitors and antibiotics
Spore morphology and location		Storage inclusions
Cellular inclusions		
Colour		
Motility		

### 2.3.1 The Bergey's Manual

In 1923, Davis Bergey, Professor of Bacteriology at the University of Pennsylvania and his colleagues published a classification of bacteria that could be used for identification of the bacterial species. The recent edition of the *Bergey's Manual of Systematic Bacteriology* not only classifies the bacteria but also provides information of the ecological characteristics of individual taxa. Table 2.3 summarizes the organization of the manual for grouping different microbial species. The five volumes of the manual describe five different groups of bacteria having both morphological and biochemical differences. These five groups are discussed in some detail in the subsequent sections.

### 2.3.2 The Archaea

Archaea are quite diverse, both in morphology and physiology and metabolically unique group of microorganisms. They can stain either gram positive or gram negative and may be spherical, rod-shaped, spiral, lobed, plate-shaped, irregularly shaped, or pleomorphic. Some are single cells whereas others form filaments or aggregates. They range in diameter from 0.1 to over 15  $\mu\text{m}$  and some filaments can grow up to 200  $\mu\text{m}$  in length. Multiplication may be by binary fission, budding, fragmentation, or other mechanisms. Archaea can be aerobic, facultatively anaerobic or strictly anaerobic. Nutritionally they range from chemolithoautotrophs to organotrophs occupying many a different types of aquatic and terrestrial environments. They are often found in extreme habitats having various types of stresses. Some are mesophiles while others are hyperthermophiles that can grow above 100°C. Recently archaea have been discovered in cold environments (See Chapter 7). It appears that they constitute up to 34% of the procaryotic biomass in coastal Antarctic surface waters. A few are symbionts in animal digestive systems.

**Table 2.2:** A comparative account of the characteristic features of bacteria, archaea and eucarya.

<i>Property</i>	<i>Bacteria</i>	<i>Archaea</i>	<i>Eucarya</i>
Nucleus with nucleolus	Absent	Absent	Present
Membranous cell organelles	Absent	Absent	Present
Cell wall	Peptidoglycan containing muramic acid	Various types without muramic acid	No muramic acid
Membrane lipids	Esterlinked straight chain fatty acids	Esterlinked branched chain fatty acids	Esterlinked straight chain fatty acids
Gas vesicles	Present	Present	Absent
t – RNA	With thymine and initiation with N-formylmethionine	No thymine and initiation with methionine	Thymine and initiation with methionine
Polysystronic m-RNA	Present	Present	Absent
m-RNA introns	Absent	Absent	Present
Ribosomes	70S	70S	80S
DNA dependent RNA polymerase structure	4 subunits	8-12 subunits	12-14 subunits
ATPase similarity	No	Yes	Yes
Methanogenesis	Absent	Present	Absent
Nitrogen fixation	Present	Present	Absent
Photosynthesis	Present	Absent	Present
Chemolithotrophy	Present	Present	Absent

Source: Prescott *et al.* (2005).

Some features of archaeal genetics are similar to those of bacteria. The archaeal chromosomes that have been studied are single closed DNA circles. However, the genomes of some archaeons are significantly smaller than the normal bacterium. For example, *E. coli* DNA has a size of about  $2.5 \times 10^9$  daltons, whereas *Thermoplasma acidophilum* DNA is about  $0.8 \times 10^9$  daltons and *Methanobacterium thermoautotrophicum* DNA is  $1.1 \times 10^9$  daltons. Archaeal mRNA appears similar to that of bacteria rather than to eucaryotic mRNA. Polygenic mRNA has been discovered and there is no evidence for mRNA splicing. Archaeal promoters are similar to those in bacteria.

Archaeal metabolism varies greatly between the members of different groups. Some archaea are organotrophs, others are autotrophic. A few even carry out an unusual form of photosynthesis. Archaeal carbohydrate metabolism probably occurs without phosphorylation of glucose in Embden-Meyerhof pathway as the enzyme 6-phosphofructokinase has not been found in archaea. Extreme halophiles and thermophiles catabolize glucose using a modified form of the Entner-Doudoroff pathway. The halophiles have slightly different modifications of the pathway than do the extreme thermophiles but still produce pyruvate and NADH or NADPH. Methanogens do not catabolize glucose to any significant extent. In

contrast with glucose degradation, gluconeogenesis proceeds by a reversal of the Embden-Meyerhof pathway in halophiles and methanogens. All archaea that have been studied can oxidize pyruvate to acetyl-CoA. They lack the pyruvate dehydrogenase complex present in eucaryotes and respiratory bacteria and use the enzyme pyruvate oxidoreductase for this purpose. Halophiles and the extreme thermophile *Thermoplasma* do seem to have a functional tricarboxylic acid cycle. No methanogen has yet been found with a complete tricarboxylic acid cycle. Evidence for functional respiratory chains has been obtained in halophiles and thermophiles.

**Table 2.3:** Classification of bacteria following the *Bergey's Manual of Systematic Bacteriology*.

<i>Taxonomic rank</i>	<i>Representative genera</i>
<b>Volume 1. The Archaea and the deeply branching and phototrophic bacteria</b>	
<b>Domain Archaea</b>	
Phylum Crenarchaeota	<i>Thermoproteus, Pyrodictium, Sulfolobus</i>
Phylum Euryarchaeota	
Class I. Methanobacteria	<i>Methanobacterium</i>
Class II. Methanococci	<i>Methanococcus</i>
Class III. Halobacteria	<i>Halobacterium, Halococcus</i>
Class IV. Thermoplasmata	<i>Thermoplasma, Picrophilus</i>
Class V. Thermococci	<i>Thermococcus, Pyrococcus</i>
Class VI. Archaeoglobi	<i>Archaeoglobus</i>
Class VII. Methanopyri	<i>Methanopyrus</i>
<b>Domain Bacteria</b>	
Phylum Aquificae	<i>Aquifex, Hydrogenobacter</i>
Phylum Thermotogae	<i>Thermotoga, Geotoga</i>
Phylum Thermodesulfobacteria	<i>Thermodesulfobacterium</i>
Phylum "Deinococcus-Thermus"	<i>Deinococcus, Thermus</i>
Phylum Chrysiogenetes	<i>Chrysogenes</i>
Phylum Chloroflexi	<i>Chloroflexus, Herpetosiphon</i>
Phylum Thermomicrobia	<i>Thermomicrobium</i>
Phylum Nitrospira	<i>Nitrospira</i>
Phylum Deferribacteres	<i>Geovibrio</i>
Phylum Cyanobacteria	<i>Synechococcus, Pleurocapsa, Anabaena</i>
Phylum Chlorobi	<i>Chlorobium, Pelodictyon</i>
<b>Volume 2. The Proteobacteria</b>	
Phylum Proteobacteria	
Class I. Alphaproteobacteria	<i>Rhodospirillum, Caulabacter, Rhizobium, Methylobacterium, Hyphomicrobium</i>
Class II. Betaproteobacteria	<i>Neisseria, Alcaligenes, Comamonas, Nitrosomonas, Methylophilus</i>
Class III. Gammaproteobacteria	<i>Chromatium, Pseudomonas, Azotobacter, Escherichia, Klebsiella, Proteus, Salmonella</i>
Class IV. Deltaproteobacteria	<i>Desulfovibrio, Bdellovibrio, Myxococcus</i>
Class V. Epsilonproteobacteria	<i>Campylobacter, Helicobacter</i>

**Table 2.3:** (Contd.)

<b>Volume 3. The low G + C gram-positive bacteria</b> Phylum <i>Firmicutes</i> Class I. Clostridia Class II. Mollicutes Class III. Bacilli	<i>Clostridium, Eubacterium, Heliobacterium</i> <i>Mycoplasma, Ureaplasma, Spiroplasma</i> <i>Bacillus, Lactobacillus, Streptococcus</i>
<b>Volume 4. The high G + C gram-positive bacteria</b> Phylum <i>Actinobacteria</i> Class Actinobacteria	<i>Actinomyces, Arthrobacter,</i> <i>Corynebacterium</i>
<b>Volume 5. The Planctomycetes, Spirochaetes, Fibrobacteres, Bacteroidetes and Fusobacteria</b> Phylum <i>Planctomycetes</i> Phylum <i>Chlamydiae</i> Phylum <i>Spirochaetes</i> Phylum <i>Fibrobacteres</i> Phylum <i>Acidobacteria</i> Phylum <i>Bacteroidetes</i>  Phylum <i>Fusobacteria</i> Phylum <i>Verrucomicrobia</i> Phylum <i>Dictyoglomus</i>	<i>Planctomyces, Gemmata</i> <i>Chlamydia</i> <i>Spirochaeta, Borrelia, Treponema</i> <i>Fibrobacter</i> <i>Acidobacterium</i> <i>Bacteroides, Porphyromonas, Prevotella,</i> <i>Flavobacterium, Sphingobacterium</i> <i>Fusobacterium, Streptobacillus</i> <i>Verrucomicrobium</i> <i>Dictyoglomus</i>

Source: Garrity (2001).

The biosynthetic pathways for amino acids, purines and pyrimidines in the archaea are similar to those in other organisms. Some methanogens can fix atmospheric dinitrogen. Not only do many archaea use a reversal of the Embden-Meyerhof pathway to synthesize glucose, but at least some methanogens and extreme thermophiles employ glycogen as their major reserve material. Autotrophy is widespread among the methanogens and extreme thermophiles and CO<sub>2</sub> fixation occurs by the reductive tricarboxylic acid cycle (*Thermoproteus*) or by the reductive acetyl-CoA pathway (*Methanococcus*). Archaeal protein synthesis has both bacterial and eucaryotic features.

Many of the archaea constitute a significant fraction of the oceanic picoplankton. They have been divided in five major groups based on physiological and morphological differences. Table 2.4 summarizes some characteristics of these five groups and gives representatives of each. The *Bergey's Manual* divides the archaea into the phyla *Euryarchaeota* and *Crenarchaeota*. The former occupy many different ecological niches and have a variety of metabolic patterns. This phylum contains seven classes, viz., *Methanobacteria*, *Methanococci*, *Halobacteria*, *Thermoplasmata*, *Thermococci*, *Archaeoglobi* and *Methanopyri*. These classes are further divided into 9 orders and 16 families. The methanogens, extreme halophiles, sulphate reducers and many extreme thermophiles with sulphur dependent

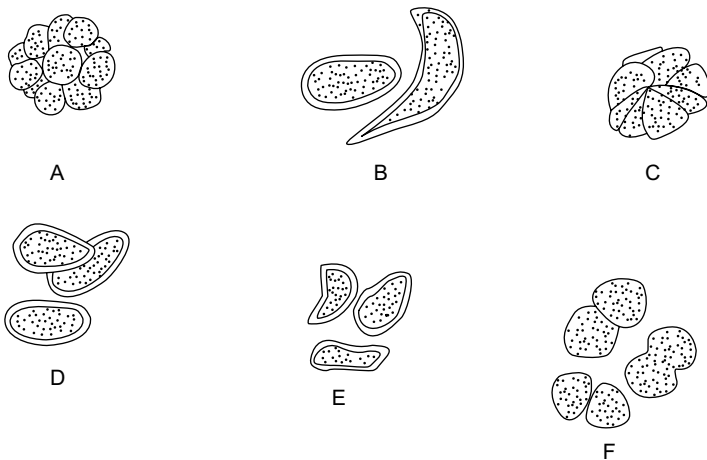
metabolism are placed in the *Euryarchaeota*. Methanogens are the dominant physiological groups (Fig. 2.3). The crenarchaeotes are thought to resemble the ancestor of the archaea and almost all the well-characterized species are thermophiles or hyperthermophiles. The phylum *Crenarchaeota* is divided into one class, *Thermoprotei*, three orders and five families. The order *Thermoproteales* contains gram-negative anaerobic to facultative, hyperthermophilic rods. They often grow chemolithoautotrophically by reducing sulphur to hydrogen sulphide. Members of the order *Sulfolobales* are coccus-shaped thermoacidophiles. The order *Desulfurococcales* contains gram-negative coccoid or disk-shaped hyperthermophiles. They grow either chemolithotrophically by hydrogen oxidation or organotrophically by either fermentation or respiration with sulphur as the electron acceptor.

**Table 2.4:** The characteristic features of the major archaeal groups.

Group	General characteristics	Representative genera
Methanogenic archaea	Strict anaerobes. Methane is the major metabolic end product. $S^0$ may be reduced to $H_2S$ without yielding energy. Cells possess coenzyme M, factors 420 and 430 and methanopterin	<i>Methanobacterium</i> <i>Methanococcus</i> <i>Methanomicrobium</i> <i>Methanosarcina</i>
Archaea sulphate reducers	Irregular gram-negative coccoid cells. $H_2S$ formed from thiosulphate and sulphate. Autotrophic growth with thiosulphate and $H_2$ . Can grow heterotrophically. Traces of methane also formed. Extremely thermophilic and strictly anaerobic. Possess factor 420 and methanopterin but not coenzyme M or factor 430	<i>Archaeoglobus</i>
Extremely halophilic archaea	Coccoid or irregularly shaped rods. Gram-negative or gram-positive, primarily aerobic chemoorganotrophs. Require high sodium chloride concentrations for growth (= 1.5 M). Neutrophilic or alkalophilic, mesophilic or slightly thermophilic. Some species contain bacteriorhodopsin and use light for ATP synthesis	<i>Halobacterium</i> <i>Halococcus</i> <i>Natronobacterium</i>
Cell wall-less archaea	Pleomorphic cells lacking a cell wall. Thermoacidophilic and chemoorganotrophic. Facultatively anaerobic. Plasma membrane contains a mannose-rich glycoprotein and a lipoglycan	<i>Thermoplasma</i>
Extremely thermophilic $S^0$ metabolizers	Gram-negative rods, filaments or cocci. Obligately thermophilic. Usually strict anaerobes but may be aerobic or facultative. Acidophilic or neutrophilic. Autotrophic or heterotrophic. Most are sulphur metabolizers. $S^0$ reduced to $H_2S$ anaerobically; $H_2S$ or $S^0$ oxidized to $H_2SO_4$ aerobically	<i>Desulfurococcus</i> <i>Pyrodictium</i> <i>Pyrococcus</i> <i>Sulfolobus</i> <i>Thermococcus</i> <i>Thermoproteus</i>

Compiled from Olsen and Woese (1997); Bell and Jackson (1998); Vetriani and Reysenbach (2000).

**(a) Phylum Crenarchaeota:** Most are extremely thermophiles and many are acidophiles and sulphur dependent. The sulphur may be used either as an electron acceptor in anaerobic respiration or as an electron source by lithotrophs. Almost all are strict anaerobes growing geothermally in heated water or soils that contain elemental sulphur. Representative genera are *Thermoproteus*, *Pyrodictium* and *Sulfolobus*.



**Figure 2.3** - Morphological variation in archaeobacteria: (A) *Sulfolobus* sp., (B) *Thermoproteus* sp., (C) *Methanasarcina* sp., (D) *Methanobrevibacterium* sp., (E) *Methanogenium* sp. and (F) *Halococcus* sp.

**(b) Phylum Euryarchaeota:** It is further divided in the following groups.

**(i) The Methanogens:** This is the largest group of archaea. These are strict anaerobes that can convert  $\text{CO}_2$ ,  $\text{H}_2$ , formate, methanol, acetate and other compounds to either methane or methane and  $\text{CO}_2$ . There are five orders, viz., *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales* and *Methanopyrales*, which differ greatly in overall shape. One of the most unusual methanogenic groups is the class *Methanopyri*, which is extremely thermophilic rod isolated from a marine hydrothermal vent. The representative genera are *Methanococcus*, *Methanothermus*, *Methanomicrobium* and *Methanogenium*.

**(ii) The Halobacteria:** This group includes extreme halophiles having 15 genera in one family. They are aerobic chemoheterotrophs with respiratory metabolism and require complex nutrients, usually proteins and amino acids, for growth. These prokaryotes have an absolute dependence on a high concentration of NaCl (at least 1.5 M). The representative genera are *Halobacterium* and *Halococcus*.

**(iii) The Thermoplasma:** These are thermoacidophiles that lack cell walls but plasma membrane is strengthened by large quantities of diglycerol tetraethers, lipopolysaccharides and glycoproteins. At present, there are three genera:

*Thermoplasma*, *Picrophilus* and *Ferroplasma*. They are sufficiently different from one another and placed in separate families. They grow in refuse piles of coal mines. These piles contain large amounts of iron pyrite (FeS), which is oxidized to sulphuric acid by chemolithotrophic bacteria. As a result the piles become very hot and acidic. This is an ideal habitat for the thermoplasmas since they grow in the temperature range between 45–65°C and pH range between 0.7 – 3.

**(iv) Sulphate-Reducing Archaea:** These are gram-negative and irregular coccoid cells with walls consisting of glycoprotein subunits. *Archaeoglobus* is extremely thermophilic (the optimum is about 83°C) and can be isolated from marine hydrothermal vents.

### 2.3.3 The Gram-Negative Nonproteobacteria

**(a) Phyla Aquificae and Thermotogae:** Two of the best-studied genera of the phylum *Aquificae* are *Aquifex* and *Hydrogenobacter*. These are gram-negative, microaerophilic rods growing in hyperthermophilic conditions with a temperature optimum of 85°C and a maximum of 95°C. *Aquifex* is an autotroph and generates energy by oxidizing donors such as hydrogen, thiosulphate and sulphur with oxygen as the acceptor. The members of the *Thermotogae* are gram-negative hyperthermophiles with a growth optimum of 80°C and a maximum of 90°C. They grow in active geothermal areas, both marine hydrothermal systems and terrestrial sulphataric springs.

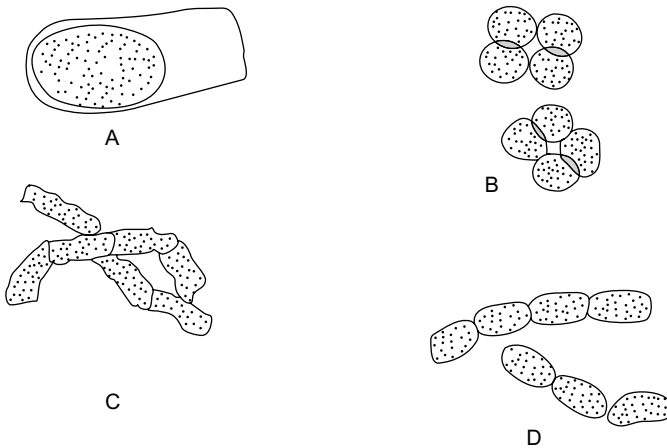
**(b) Phylum Deinococcus-Thermus:** It contains only three genera with *Deinococcus* being best studied one. Deinococci are spherical or rod-shaped aerobic and mesophilic bacteria and can produce acid from only a few sugars. Although they stain gram positive, their cell wall is layered and has an outer membrane like the gram-negative bacteria. Almost all strains are extraordinarily resistant to both desiccation and radiation and can survive as much as 3 to 5 million rad of radiation.

**(c) Photosynthetic bacteria:** There are three groups of gram-negative photosynthetic bacteria: the purple bacteria, the green bacteria and the cyanobacteria. The cyanobacteria differ most fundamentally from the green and purple photosynthetic bacteria in being able to carry out oxygenic photosynthesis. They use water as an electron donor and generate oxygen during photosynthesis. In contrast, purple and green bacteria use anoxygenic photosynthesis. Because they are unable to use water as an electron source, they employ reduced molecules such as hydrogen sulphide, sulphur, hydrogen and organic matters as their electron source for the generation of NADH and NADPH. Consequently, purple and green bacteria do not produce oxygen but many form sulphur granules. Purple sulphur bacteria accumulate granules within their cells, whereas green sulphur bacteria deposit the sulphur granules outside their cells. The purple nonsulphur bacteria normally use organic molecules as an electron source. There



are also differences in photosynthetic pigments, the organization of photosynthetic membranes, nutritional requirements and oxygen relationships among different photosynthetic bacteria (Table 2.5).

The *Bergey's Manual* places photosynthetic bacteria into seven major groups distributed among five bacterial phyla. The phylum *Chloroflexi* contains the green nonsulphur bacteria and the phylum *Chlorobi* the green sulphur bacteria. The cyanobacteria are placed in their own phylum, *Cyanobacteria*. Purple bacteria are divided in three groups. Purple sulphur bacteria are placed in the  $\gamma$ -proteobacteria. The purple nonsulphur bacteria are distributed between the  $\alpha$ -proteobacteria and  $\beta$ -proteobacteria. Finally, the gram-positive heliobacteria in the phylum *Firmicutes* are also photosynthetic having different structural peculiarities (Fig. 2.4).



**Figure 2.4** - The morphological variations in non-proteobacteria. (A) *Thermotoga maritima* showing extended loose seath, (B) *Deinococcus* sp., (C) *Pelodictyon* sp. and (D) *Chlorobium* sp.

**(d) Phylum Spirochaetes:** This phylum contains gram-negative, chemo-heterotrophic bacteria distinguished by their structure and mechanism of motility. They are slender, long bacteria (0.1 to 3.0  $\mu\text{m}$  by 5 to 250  $\mu\text{m}$ ) with a flexible, helical shape. Spirochaetes differ greatly from other bacteria with respect to motility and can move through very viscous solutions though they lack external rotating flagella. They can be anaerobic, facultatively anaerobic or aerobic. Carbohydrates, amino acids, long-chain fatty acids and long-chain fatty alcohols may serve as carbon and energy sources. This group of bacteria is exceptionally diverse ecologically and grows in habitats ranging from mud to the human mouth. Members include the genera *Spirochaeta*, *Leptospira*, *Trepollema* and *Borrelia*.

**Table 2.5:** Characteristics of the major groups of gram-negative photosynthetic bacteria.

Characteristics	Green sulphur	Green non-sulphur	Purple sulphur	Purple non-sulphur
Major photosynthetic pigments	Bacteriochlorophylls <i>a</i> plus <i>c</i> , <i>d</i> , or <i>e</i>	Bacteriochlorophylls <i>a</i> and <i>c</i>	Bacteriochlorophylls <i>a</i> and <i>b</i>	Bacteriochlorophylls <i>a</i> and <i>b</i>
Morphology of photosynthetic membranes	Photosynthetic system partly in chlorosomes that are independent of the plasma membrane	Chlorosomes present when grown anaerobically	Photosynthetic system contains in spherical or lamellar membrane complexes that are continuous with the plasma membrane	Photosynthetic system contained in spherical or lamellar membrane complexes that are continuous with the plasma membrane
Photosynthetic electron donors	H <sub>2</sub> , H <sub>2</sub> S, S	H <sub>2</sub> , H <sub>2</sub> S	H <sub>2</sub> , H <sub>2</sub> S, S	Usually organic molecules; some times reduced sulphur compounds or H <sub>2</sub>
Sulphur deposition	Outside of the cell	None	Inside of the cell	Outside of the cell in few cases
General metabolic type	Obligately anaerobic	Usually photoheterotrophic; sometimes photoautotrophic or chemoheterotrophic	Obligately anaerobic photolithoautotrophs	Usually anaerobic photoorganoheterotrophs

**(e) Phylum Bacteroidetes:** The members of this phylum are anaerobic, gram-negative, nonsporing, motile or nonmotile rods of various shapes. These bacteria are chemoheterotrophic and usually produce a mixture of organic acids as fermentation end products, but they do not reduce sulphate or other sulphur compounds. These bacteria grow in habitats such as the oral cavity and intestinal tract of humans and other animals and the rumen. About 30% of the bacteria isolated from human feces are members of the genus *Bacteroides* and these organisms may provide extra nutrition by degrading cellulose, pectins and other complex carbohydrates. They are also involved in human diseases. Members of the genus *Bacteroides* are associated with diseases of major organ systems, ranging from the central nervous system to the skeletal system. Important genera are *Sphingobacterium*, *Saprospira*, *Flexibacter*, *Cytophaga*, *Sporocytophaga* and *Crenothrix*.

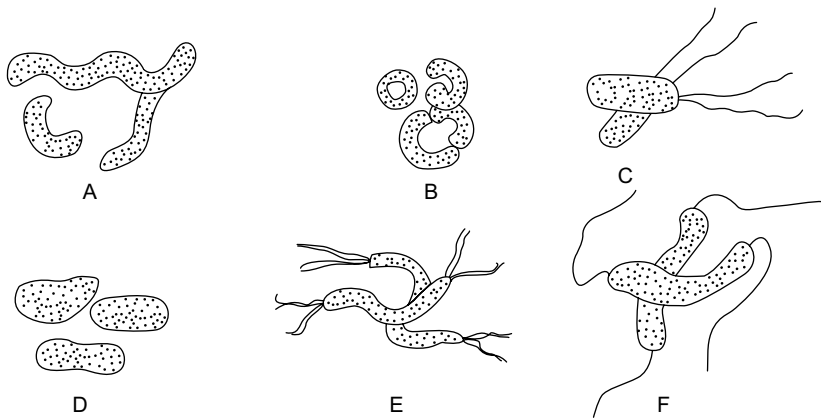
### 2.3.4 The Proteobacteria

The proteobacteria are highly diverse in their phenotypic properties, nutritional type, oxygen relationship and motility. Many of these bacteria are gram-negative with ability to accommodate themselves to a variety of habitats. They are very useful experimental tools in many laboratories. Although many of these bacteria

do not vary drastically in general appearance, they often are very diverse in their metabolism and life style, which range from obligate intracellular parasitism to a free living existence in soil and aquatic habitats (Table 2.6).

**(a)  $\alpha$ -Proteobacteria:** They include most of the oligotrophic proteobacteria. Some have unusual metabolic modes such as methylotrophy (*Methylobacterium*), chemolithotrophy (*Nitrobacter*) and the ability to fix nitrogen (*Rhizobium*). Members of genera such as *Rickettsia* and *Brucella* are important pathogens. In fact, *Rickettsia* has become an obligately intracellular parasite. Many genera are characterized by distinctive morphology such as prosthecae (Fig. 2.5).

- (i) The purple nonsulphur bacteria: All the purple bacteria use anoxygenic photosynthesis, possess bacteriochlorophylls *a* or *b* and have their photosynthetic apparatus in membrane systems that are continuous with the plasma membrane. Normally they grow anaerobically as photoorganoheterotrophs (Table 2.5). In dark, most purple nonsulphur bacteria can grow aerobically as chemoorganoheterotrophs, but some species carry out fermentation and grow anaerobically.



**Figure 2.5** - The morphological variation in proteobacteria. (A) *Rhodospirillum* sp., (B) *Rhodocyclus* sp., (C) *Rhizobium* sp., (D) *Nitrosomonas* sp., (E) *Spirillum* sp. and (F) *Thiomicrospira* sp.

- (ii) Nitrogen fixing bacteria: This group includes at least 10 families with a great variation of phenotypes. The gram-negative, aerobic genera *Rhizobium* and *Agrobacterium* are the two well studied genera. Members of the genus *Rhizobium* are motile rods, often containing poly- $\beta$ -hydroxybutyrate granules, that become pleomorphic under adverse conditions. They grow symbiotically within root nodule cells of legumes as nitrogen-fixing bacteroids. In contrast, *Azotobacter* is a free-living soil genus and fixes atmospheric nitrogen nonsymbiotically.
- (iii) Nitrifying bacteria: The taxonomy of these aerobic chemolithotrophic bacteria, those derive energy and electrons from reduced inorganic

compounds, is quite complex. Normally these bacteria use CO<sub>2</sub> as their carbon source and thus are chemolithoautotrophs, but some can function as chemolithoheterotrophs and use reduced organic carbon sources. The nitrifying bacteria are  $\alpha$ -,  $\beta$ - and  $\gamma$ -proteobacteria type. *Nitrobacter* is  $\alpha$ -proteobacteria; *Nitrosomonas* and *Nitrosospira* are  $\beta$ -proteobacteria; *Nitrococcus* and *Nitrosococcus* are  $\gamma$ -proteobacteria.

**Table 2.6:** The commonly occurring genera of gram-negative proteobacteria.

$\alpha$ -proteobacteria	$\beta$ -proteobacteria	$\gamma$ -proteobacteria	$\delta$ -proteobacteria	Nitrifying bacteria
<i>Agrobacterium</i>	<i>Bordetella</i>	<i>Azotobacter</i>	<i>Bdellovibrio</i>	<i>Nitrosomonas</i>
<i>Caulobacter</i>	<i>Burkholderia</i>	<i>Beggiatoa</i>	<i>Desulfovibrio</i>	<i>Nitrococcus</i>
<i>Hyphomicrobium</i>	<i>Leptothrix</i>	<i>Chromatium</i>	<i>Desulfuromonas</i>	<i>Nitrosospira</i>
<i>Nitrobacter</i>	<i>Neisseria</i>	<i>Ectothiorhodospira</i>	<i>Myxococcus</i>	<i>Nitrosococcus</i>
<i>Rhizobium</i>	<i>Nitrosomonas</i>	<i>Escherichia</i>	<i>Stigmatella</i>	<i>Nitrobacter</i>
<i>Rhodospirillum</i>	<i>Sphaerotilus</i>	<i>Haemophilus</i>		
<i>Rickettsia</i>	<i>Thiobacillus</i>	<i>Leucothrix</i>		
		<i>Methylococcus</i>		
		<i>Photobacterium</i>		
		<i>Pseudomonas</i>		
		<i>Vibrio</i>		

**(b)  $\beta$ -Proteobacteria:** The  $\beta$ -proteobacteria overlap the  $\alpha$ -proteobacteria metabolically but tend to use substances that diffuse from organic decomposition in the anaerobic zone of habitats. Some of these bacteria use hydrogen, ammonia, methane, volatile fatty acids and similar substances. The  $\beta$ -proteobacteria may be chemoheterotrophs, photolithotrophs, methylotrophs and chemolithotrophs. The subgroup contains two genera with important human pathogens: viz., *Neisseria* and *Bordetella*. The other important genera of this group are *Burkholderia*, *Nitrosomonas*, *Nitrosospira*, *Gallionella* and *Spirillum*. The genus *Thiobacillus* is one of the best-studied chemolithotrophs and most prominent of the colourless sulphur bacteria. Like the nitrifying bacteria, colourless sulphur bacteria are a highly diverse group. Many are unicellular rod-shaped or spiral sulphur-oxidizing bacteria that are nonmotile or motile by flagella.

Sulphur-oxidizing  $\beta$ -proteobacteria have wide distribution and great practical importance. *Thiobacillus* grows in soil and aquatic habitats, both freshwater and marine. In marine habitats *Thiomicrospira* is more important than *Thiobacillus*. Because of their great acid tolerance, these bacteria prosper in habitats they have acidified by sulphuric acid production (pH as low as 0.5), even though most other organisms are dying. The production of large quantities of sulphuric acid and ferric iron by *T. ferrooxidans* corrodes concrete and pipe structures (See Chapter 11). Thiobacilli often cause extensive acid and metal pollution when they release metals from mine wastes. However, sulphur-oxidizing bacteria are also beneficial. They may increase soil fertility when they release elemental sulphur by oxidizing it to sulphate. Thiobacilli are used in processing low-grade metal ores because of their ability to leach metals from ore (See Chapter 13).

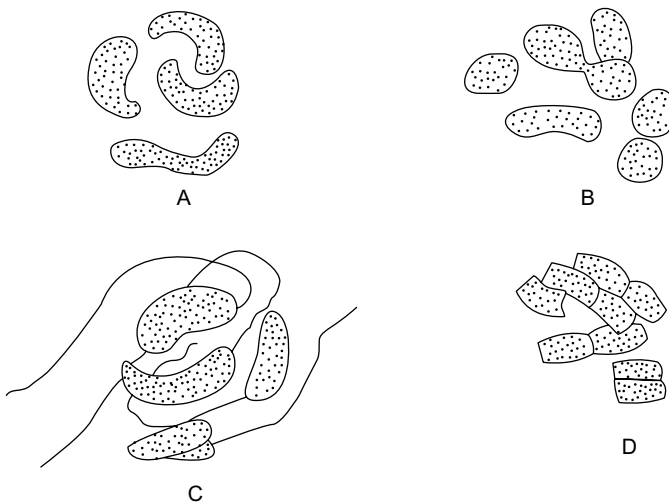
**(c)  $\gamma$ -Proteobacteria:** The  $\gamma$ -proteobacteria constitute the largest subgroup of proteobacteria with an extraordinary variety of physiological types. Many important genera are chemoorganotrophic and facultatively anaerobic. Other genera contain aerobic chemoorganotrophs, photolithotrophs, chemolithotrophs or methylotrophs. According to some DNA-rRNA hybridization studies, the  $\gamma$ -proteobacteria are composed of several deeply branching groups. One consists of the purple sulphur bacteria; a second includes the intracellular parasites *Legionella* and *Coxiella*. The two largest groups contain a wide variety of nonphotosynthetic genera. Many genera of the group are facultative anaerobes and use the glycolytic and pentose phosphate pathways to catabolize carbohydrates. The genera *Pseudomonas*, *Azotobacter*, *Moraxella*, *Xanthomonas* and *Acinetobacter* belong to this superfamily.

The family *Methylococcaceae*, belonging to this group of bacteria, contains rods, vibrios and cocci that use methane, methanol and other reduced one-carbon compounds as their sole carbon and energy sources under aerobic or microaerobic (low oxygen) conditions, i.e. they are methylotrophs. The family contains seven genera, two of which are *Methylococcus* (spherical, nonmotile cells) and *Methylomonas* (straight, curved, or branched rods with a single, polar flagellum). When oxidizing methane, the bacteria contain complex arrays of intracellular membranes. Almost all can form some types of resting stages, often a cyst, somewhat like that of the azotobacteria. Methylotrophic growth depends on the presence of methane and related compounds. Methanogenesis from substrates such as  $H_2$  and  $CO_2$  is widespread in anaerobic soil and water and methylotrophic bacteria grow above anaerobic habitats all over the world.

The pseudomonads are the most abundant and widely distributed members of  $\gamma$ -proteobacteria. *Pseudomonas* is the most well studied and well represented genus. It is straight or slightly curved gram-negative rod. These chemoheterotrophs are aerobic and carry out respiratory metabolism with  $O_2$  (and sometimes nitrate) as the electron acceptor. All pseudomonads have a functional tricarboxylic acid cycle and can oxidize substrates to  $CO_2$ . Most hexoses are degraded by the Entner-Doudoroff pathway rather than glycolytically. Many pseudomonads can degrade an exceptionally a wide variety of organic molecules. Thus they are very important in the mineralization process in nature and in sewage treatment. The fluorescent pseudomonads can use approximately 80 different substances as their carbon and energy sources. Some pseudomonads are major animal and plant pathogens and also are involved in the spoilage of refrigerated milk, meat, eggs and seafood because they can grow at  $4^\circ C$  and degrade lipids and proteins.

**(d)  $\delta$ -Proteobacteria:** Although the  $\delta$ -proteobacteria are not a large assemblage of genera, they show considerable morphological and physiological diversity. These bacteria can be divided in two general groups, all of them chemoorganotrophs. Some genera are predators such as the bdellovibrios and myxobacteria. Others are anaerobes that generate sulphide from sulphate and sulphur while oxidizing organic matters. Figure 2.6 illustrates the basic structures of some representative genera.

These sulphate- or sulphur-reducing bacteria are a diverse group united by their anaerobic nature and the ability to use elemental sulphur or sulphate and other oxidized sulphur compounds as electron acceptors during anaerobic respiration. An electron transport chain generates ATP and reduces sulphur and sulphate to hydrogen sulphide. This process is very important in the cycling of sulphur within the ecosystem. Because significant amounts of sulphate are present in almost all aquatic and terrestrial habitats, sulphate reducing bacteria are widespread and active in locations made anaerobic by microbial digestion of organic materials. *Desulfovibrio* and other sulphate-reducing bacteria thrive in habitats such as muds and sediments of polluted lakes and streams, sewage lagoons and digesters and waterlogged soils. Sulphate-reducing bacteria negatively impact industry by accelerating corrosion.

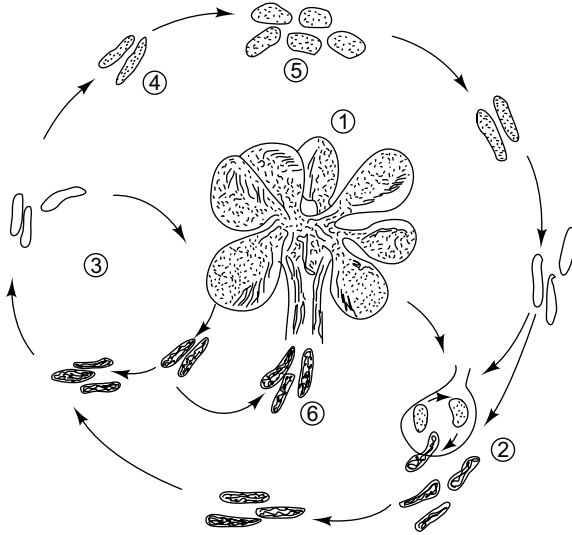


**Figure 2.6** - Morphological variations in Deltaproteobacteria: (A) *Desulfovibrio* sp., (B) *Desulfobacter* sp., (C) *Bdellovibrio* sp. and (D) *Chondromyces* sp.

*Bdellovibrio* and myxobacteria are gram-negative, aerobic soil bacteria. These are micropredators or scavengers. The latter secrete an array of digestive enzymes that lyse bacteria and also secrete antibiotics, which may kill their prey. The digestion products, primarily small peptides, are absorbed. Most myxobacteria use amino acids as their major source of carbon, nitrogen and energy. All are chemoheterotrophs with respiratory metabolism.

The myxobacterial life cycle is quite distinctive and in many ways resembles that of the cellular slime molds (Fig. 2.7). In the presence of a food supply, myxobacteria migrate along a solid surface, feeding and leaving slime trails. During this stage the cells often form a swarm and move in a coordinated fashion. Some species congregate to produce a sheet of cells that moves rhythmically to generate waves or ripples. When their nutrient supply is exhausted, the myxobacteria aggregate and differentiate into a fruiting body. This is a complex developmental process triggered by starvation and different signals given by

specific proteins and amino acids. The fruiting bodies vary in complexity from simple globular objects (*Myxococcus*) to the elaborate, branching, treelike structures (*Srigmatella*). The fruiting body develops myxospores and releases them at maturity. A mass of myxobacteria can produce enzyme concentrations sufficient to digest their prey more easily than an individual cell can.



**Figure 2.7** - The myxobacterial life cycle showing sporulation and germination processes. (1) Fruiting body from cooperative morphogenesis, (2) inversion, (3) vegetative life cycle, (4) spores from vegetative cells, (5) mixospores, (6) vegetative cells for fruiting body formation.

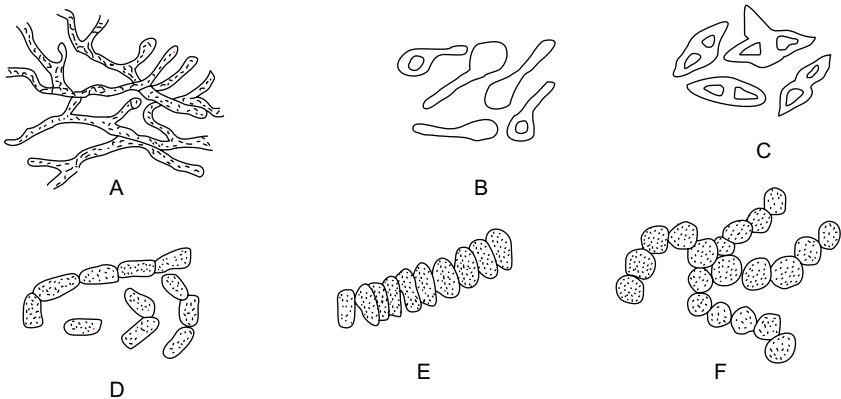
Myxobacteria are found in soils worldwide. They are most commonly isolated from neutral soils or decaying plant material such as leaves and tree bark and from animal dung. Although they grow in habitats as diverse as tropical rain forests and the arctic tundra, they are most abundant in warm areas.

**(e)  $\epsilon$ -Proteobacteria:** The  $\epsilon$ -proteobacteria are the smallest of the five proteobacterial classes. They all are slender gram-negative rods, which can be straight, curved, or helical. The two most important genera, *Campylobacter* and *Helicobacter*, are microaerophilic, motile, helical or vibrioid, gram-negative rods. The genus *Campylobacter* contains both nonpathogens and species pathogenic for humans and other animals. The species of *Helicobacter*, have been isolated from the stomachs and upper intestines of humans, dogs, cats and other mammals. In developing countries 70 to 90% of the population is infected with different species of  $\epsilon$ -proteobacteria.

### 2.3.5 The Mollicutes

Members of the class Mollicutes are commonly called mycoplasmas. These bacteria lack cell walls and cannot synthesize peptidoglycan precursors. Thus they are penicillin resistant but susceptible to lysis by osmotic shock and detergent

treatment. Because they are bounded only by a plasma membrane, these prokaryotes are pleomorphic and vary in shape from spherical or pear-shaped organisms, about 0.3 to 0.8  $\mu\text{m}$  in diameter, to branched or helical filaments (Fig. 2.8A)). Some mycoplasmas (e.g., *Mycoplasma genitalium*) have a specialized terminal structure that projects from the cell and gives them a flask or pear shape. This structure aids in attachment to eucaryotic cells. They are the smallest bacteria capable of self-reproduction. Although most are nonmotile, some can glide along liquid-covered surfaces. Most species differ from the vast majority of bacteria in requiring sterols for growth. They usually are facultative anaerobes, but a few are obligate anaerobes. When growing on agar, most species form colonies with a “fried-egg” appearance because they grow into the agar surface at the centre while spreading outward on the surface at the colony edges. Their genome is one of the smallest found in prokaryotes, about 5 to  $10 \times 10^8$  daltons: the G + C content ranges from 23 to 41%. Recently the complete genome of *M. genitalium*, a parasite of the human genital and respiratory tracts, has been sequenced.



**Figure 2.8** - The morphological variations in the low G + C containing gram-positive bacteria. (A) *Mycoplasma* sp., (B) *Clostridium* sp., (C) *Desulfotomaculum* sp., (D) *Bacillus* sp., (E) *Caryophanon* sp. and (F) *Streptococcus* sp.

The metabolism of mycoplasmas is not particularly unusual, although they are deficient in several biosynthetic sequences and often require sterols, fatty acids, vitamins, amino acids, purines and pyrimidines. Mycoplasmas are usually more osmotically stable than bacterial protoplasts and their membrane sterols may be a stabilizing factor. Some produce ATP by the Embden-Meyerhof pathway and lactic acid fermentation. Others catabolize arginine or urea to generate ATP. None appears to have the complete tricarboxylic acid cycle.

Mycoplasmas are remarkably widespread and can be isolated from animals, plants, the soil and even compost piles. Indeed, about 10% of the mammalian cell cultures in use are probably contaminated with mycoplasmas, which seriously interfere with tissue culture experiments and are difficult to detect and eliminate. In animals, mycoplasmas colonize mucous membranes and joints and often are associated with diseases of the respiratory and urogenital tracts. Spiroplasmas have been isolated from insects, ticks and a variety of plants. They cause disease



in citrus plants, cabbage, broccoli, honeybees and other hosts. Presumably many more pathogenic mollicutes will be discovered as techniques for their isolation and study improve.

### 2.3.6 Low G+C Gram-Positive Bacteria in Bergey's Manual

*Bacillus*, *Clostridium*, *Lactobacillus*, *Corynebacterium*, *Mycobacterium* and *Nocardia* are the most widely distributed genera of this group (Table 2.7). These are endospore-forming bacteria distributed widely and mostly growing as soil inhabitants. Soil conditions are often extremely variable and endospores are an obvious advantage in surviving periods of dryness or nutrient deprivation in these bacteria.

**Table 2.7:** The representative genera of some gram-positive bacteria.

<i>Mollicutes</i>	<i>Clostridia and relatives</i>	<i>Bacilli</i>	<i>Actinobacteria</i>
<i>Acholeplasma</i>	<i>Clostridium</i>	<i>Bacillus</i>	<i>Actinoplanes</i>
<i>Anaeroplasm</i>	<i>Desulfotomaculum</i>	<i>Caryophanon</i>	<i>Arthrobacter</i>
<i>Asteroleplasma</i>	<i>Heliobacterium</i>	<i>Enterococcus</i>	<i>Bifidobacterium</i>
<i>Entomoplasm</i>	<i>Veillonella</i>	<i>Lactobacillus</i>	<i>Corynebacterium</i>
<i>Mesoplasm</i>		<i>Lactococcus</i>	<i>Frankia</i>
<i>Mycoplasma</i>		<i>Leuconostoc</i>	<i>Micrococcus</i>
<i>Spiroplasma</i>		<i>Staphylococcus</i>	<i>Mycobacterium</i>
<i>Ureaplasma</i>		<i>Streptococcus</i>	<i>Nocardia</i>
		<i>Thermoactinomyces</i>	<i>Propionibacterium</i>
			<i>Streptomyces</i>

Members of the genus *Clostridium* have great practical impact. Because they are anaerobic and form heat-resistant endospores, they are responsible for many cases of food spoilage, even in canned foods. *C. botulinum* is the causative agent of botulism. Clostridia often can ferment amino acids to produce ATP by oxidizing one amino acid and using another as an electron acceptor in a process called the Stickland reaction. This reaction generates ammonia, hydrogen sulphide, fatty acids and amines during the anaerobic decomposition of proteins. These products are responsible for many unpleasant odours arising during putrefaction. Several clostridia produce toxins and are major disease causing agents.

In addition to the genus *Clostridium*, several other genera of this group are also environmentally important. For example, *Desulfotomaculum* reduces sulphate and sulphite to hydrogen sulphide during anaerobic respiration. Although it stains gram-negative, electron microscopic studies have shown that *Desulfotomaculum* has a gram-positive type cell wall despite its staining characteristics and really is a member of the low G + C gram positives. The heliobacteria, genera *Heliobacterium* and *Heliophilum*, are a group of unusual anaerobic photosynthetic bacteria characterized by the presence of bacteriochlorophyll *g*. They have a photosystem I type reaction centre like the green sulphur bacteria, but have no intracytoplasmic photosynthetic membranes. The pigments are contained in the plasma membrane.

The genus *Bacillus* contains gram-positive, endospore-forming, chemoheterotrophic rods that are usually motile and peritrichously flagellated. It is aerobic, or sometimes facultative and catalase positive (Table 2.8). Many species have been placed in new families and genera based on rRNA sequence data. Some examples of organisms that were formerly in the genus *Bacillus* are *Paenibacillus* and *Alicyclobacillus*. Many species of *Bacillus* are of considerable importance. For example, members of the genus *Bacillus* produce the antibiotics bacitracin, gramicidin and polymyxin. *B. cereus* causes some forms of food poisoning and can infect humans. *B. anthracis* is the causative agent of the disease anthrax, which can affect both farm animals and humans. Several species (*B. thuringiensis* and *B. sphaericus*) are used as insecticides.

The most important genus *Staphylococcus* is facultatively anaerobic, nonmotile, gram-positive cocci that usually form irregular clusters. Staphylococci are normally associated with the skin, skin glands and mucous membranes of warm-blooded animals and are responsible for many human diseases. *S. epidermidis* is a common skin resident that is sometimes responsible for endocarditis and infections of patients with lowered resistance (e.g., wound infections, surgical infections, urinary tract infections). *S. aureus* is the most important human staphylococcal pathogen and causes boils, abscesses, wound infections, pneumonia, toxic shock syndrome and other diseases. It is also a major cause of food poisoning.

**Table 2.8:** The list of important genera of bacteria on the basis of their choice of oxygen relationship.

<i>Aerobic</i>	<i>Aerobic to microaerophilic</i>	<i>Facultative anaerobic</i>	<i>Anaerobic</i>
<i>Leptospira</i>	<i>Treponema</i>	<i>Spirochaeta</i>	<i>Brachyspira</i>
<i>Nitrobacter</i>	<i>Borrelia</i>	<i>Cristispira</i>	<i>Serpulina</i>
<i>Rhizobium</i>	<i>Rhodospirillum</i>	<i>Escherichia</i>	<i>Chromatium</i>
<i>Caulobacter</i>	<i>Beggiatoa</i>	<i>Haemophilus</i>	<i>Ectothirodospira</i>
<i>Agrobacterium</i>	<i>Campylobacter</i>	<i>Photobacterium</i>	<i>Methanococcus</i>
<i>Burkholderia</i>	<i>Helicobacter</i>	<i>Vibrio</i>	<i>Methanospirillum</i>
<i>Leptothrix</i>	<i>Lactobacillus</i>	<i>Bacillus</i>	<i>Desulfovibrio</i>
<i>Nitrosomonas</i>	<i>Frankia</i>	<i>Enterococcus</i>	<i>Desulfuromonas</i>
<i>Thiobacillus</i>		<i>Lactococcus</i>	<i>Methanogenium</i>
<i>Azotobacter</i>		<i>Leuconostoc</i>	<i>Clostridium</i>
<i>Methylococcus</i>		<i>Staphylococcus</i>	<i>Heleobacterium</i>
<i>Pseudomonas</i>		<i>Streptococcus</i>	<i>Bifidobacterium</i>
<i>Bacillus</i>		<i>Corynebacterium</i>	
<i>Bdellovibrio</i>			
<i>Myxococcus</i>			
<i>Stigmatella</i>			
<i>Streptomyces</i>			

Compiled from Amesz (1995); Takeuchi *et al.* (1995); Hanson and Hanson (1996); Radoff *et al.* (1999); Brun (2000); Hooykaas (2000); Margulis (2000).

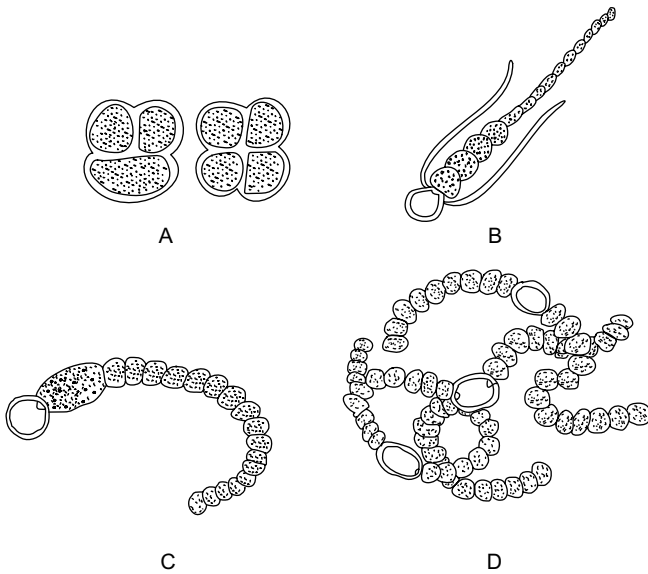
blue-green because of phycocyanin, a few cyanobacteria (e.g. *Calothrix*) are red or brown in colour because of the dominance of red pigment phycoerythrin (Table 2.9). Despite this variety, cyanobacteria have typical procaryotic cell structures and a normal gram-negative type cell wall. They often use gas vesicles to move vertically in the water and many filamentous species have gliding motility. Although cyanobacteria lack flagella, several strains of the marine genus *Synechococcus* are able to move at rates of up to 25  $\mu\text{m}/\text{second}$  by means of an unknown mechanism.

**Table 2.9:** Characteristics of the cyanobacterial subsections used for their taxonomic separation.

<i>Subsection</i>	<i>General shape and growth</i>	<i>Reproduction</i>	<i>Other properties</i>	<i>Common genera</i>
I	Unicellular rods or cocci; nonfilamentous aggregates	Binary fission, budding	Almost always nonmotile	<i>Chamaesiphon</i> <i>Chroococcus</i> <i>Gloethece</i> <i>Gleocapsa</i>
II	Unicellular rods or cocci; may be held together in aggregates	Multiple fission to form bacocytes	Only some bacocytes are motile	<i>Pleurocapsa</i> <i>Dermocarpa</i> <i>Chroococciopsis</i>
III	Filamentous, unbranched trichome with only vegetative cells	Binary fission in a single plane, fragmentation	Usually motile	<i>Lyngbya</i> <i>Oscillatoria</i> <i>Prochlorothrix</i> <i>Spirulina</i> <i>Pseudoanabaena</i>
IV	Filamentous, unbranched trichome may contain specialized cells (heterocysts)	Binary fission in a single plane, fragmentation to form hormogonia	Often motile, may produce akinetes	<i>Anabaena</i> <i>Cylindrospermum</i> <i>Aphanizomenon</i> <i>Nostoc</i> <i>Scytonema</i> <i>Calothrix</i>
V	Filamentous trichomes either with branches or composed of more than one row of cells	Binary fission in more than one plane; hormogonia formed	May produce akinetes; greatest morphological complexity and differentiation in cyanobacteria	<i>Fischerella</i> <i>Stigonema</i> <i>Geitleria</i>

Compiled from Adams (1992); Garcia-Pichel (2000); Raymond *et al.* (2002).

Cyanobacteria show great diversity with respect to reproduction and employ a variety of mechanisms: viz., binary fission, budding, fragmentation and multiple fission. Fragmentation of filamentous cyanobacteria can generate small, motile filaments called hormogonia. Some species develop akinetes, specialized, dormant, thick-walled resting cells that are resistant to desiccation.



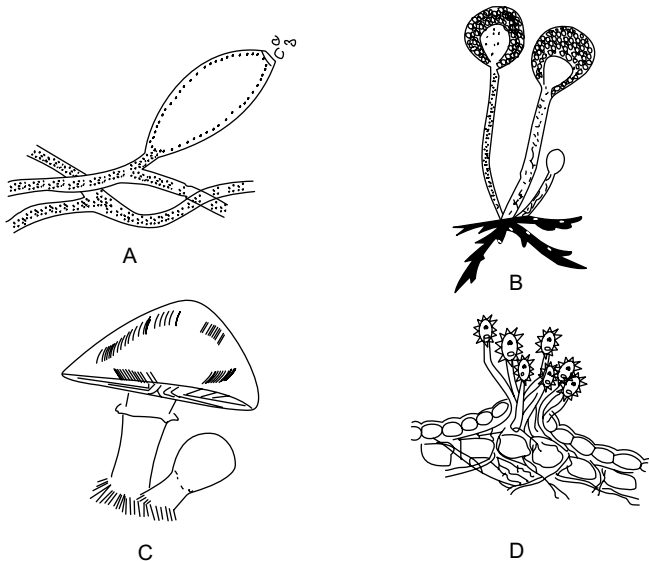
**Figure 2.10** - Representatives of cyanobacteria. (A) *Chroococcus* sp., (B) *Rivularia* sp., (C) *Calothrix* sp. and (D) *Nostoc* sp.

Many filamentous cyanobacteria fix atmospheric nitrogen by means of special cells called heterocysts. Around 5 to 10% of the cells can develop into heterocysts when cyanobacteria are deprived of both nitrate and ammonia, their preferred nitrogen sources. This can go up to 20% under extreme nitrogen deficient conditions and still higher when the cyanobacteria are in symbiotic association with a host that supply metabolites to the microbial symbiont. When transforming themselves into heterocysts, cyanobacterial cells synthesize a very thick new wall, reorganize their photosynthetic membranes, discard their phycobiliproteins and photosystem II and synthesize the nitrogen-fixing enzyme nitrogenase. Photosystem I is still functional and produces ATP, but no oxygen arises from noncyclic photophosphorylation because photosystem II is absent. This inability to generate  $O_2$  is critical because the nitrogenase is extremely oxygen sensitive. The heterocyst wall slows or prevents  $O_2$  diffusion into the cell and any  $O_2$  present is consumed during respiration for its own maintenance. The structure and physiology of the heterocyst ensures that it will remain anaerobic; it is dedicated to nitrogen fixation. It obtains nutrients from adjacent vegetative cells and contributes fixed nitrogen in the form of the amino acid glutamine. Some nonheterocystous cyanobacteria also fix nitrogen when are present under dark, anoxic conditions in microbial mats. Planktonic forms such as *Trichodesmium* can fix nitrogen as well.

The classification of cyanobacteria is still in an unsettled state, partly due to the lack of pure cultures. The second edition of *Bergey's Manual* divides the cyanobacteria into five subsections and separates Prochlorophytes from the cyanobacteria. The prochlorophytes are oxygenic phototrophs that have both

## 2.5.2 Morphological Complexity

The vegetative thallus varies in complexity and size, ranging from the single-cell microscopic yeasts to multicellular molds, macroscopic puffballs and mushrooms (Fig. 2.11). The fungal cell is usually enclosed in a cell wall of chitin, which is a strong but flexible nitrogen-containing polysaccharide consisting of N-acetylglucosamine residues. A yeast is a unicellular fungus with cells that are larger than bacteria, vary considerably in size and are commonly spherical to egg shaped. They have no flagella but do possess most of the other eucaryotic organelles. A mold consists of long, branched, thread-like filamentous hyphae that form a mycelium. In some fungi, protoplasm streams through hyphae, uninterrupted by cross walls (coenocytic). The hyphae of other fungi are septate with either a single pore or multiple pores in the septa that permit cytoplasmic streaming. Many fungi, especially those that cause diseases in humans and animals, are dimorphic. The latter can change from (1) the yeast (Y) form in the animal to (2) the mold or mycelial form (M) in the external environment in response to changes in various environmental factors (nutrients, CO<sub>2</sub> tension, oxidation-reduction potentials, temperature). This shift is called the YM shift. In plant associated fungi the opposite type of dimorphism exists: the mycelial form occurs in the plant and the yeast form in the external environment.



**Figure 2.11** - Morphology of some fungi with their spore producing structures. (A) *Saprolegnia* sp., (B) *Rhizopus* sp., (C) *Agaricus* sp., (D) *Puccinia* uredial structure.

## 2.5.3 Fungal Nutrition

Fungi grow best in dark, moist habitats, but they are found wherever organic material is available. Most fungi are saprophytes, securing their nutrients from

**(c) Division Basidiomycota:** This division contains the basidiomycetes, commonly known as the club fungi. These are named for their characteristic structure of cell, the basidium, that is involved in sexual reproduction. A basidium is produced at the tip of hyphae and normally is club shaped. Two or more basidiospores are produced by the basidium and basidia may be held within fruiting bodies called basidiocarps. Examples of this division include the smuts, jelly fungi, rusts, shelf fungi, stinkhorns, puffballs, toadstools, mushrooms and bird's nest fungi.

Most basidiomycetes are saprophytes that decompose plant debris, especially cellulose and lignin. Many mushrooms are used as food throughout the world. Different species of *Agaricus* are edible and are cultivated widely to supplement the world food demand. Many mushrooms also produce specific alkaloids that act as either poisons or hallucinogens. One such example is the “destroying angel” mushroom, *Amanita phalloides*, which contains hepatotoxins phalloidin and  $\alpha$ -amanitin. The commonly occurring genera of the division basidiomycota are *Puccinia*, *Ustilago*, *Agaricus*, etc.

**(d) Division Deuteromycota:** The fungi, that lacks a well defined sexual phase (perfect stage), or if this phase has not yet been observed, are placed within the division *Deuteromycota*, commonly called the Fungi Imperfecti or deuteromycetes (“secondary fungi” or mitosporic fungi). Most deuteromycetes reproduce by means of conidia. Once a perfect stage is observed, the fungus is transferred to its proper division (often to the *Ascomycota*). Molecular systematics places the *Deuteromycota* among their closest relatives in the *Eumycota*. Most Fungi Imperfecti are terrestrial, with only a few being reported from freshwater and marine habitats. The majority are either saprophytes or parasites of plants. A few are parasitic on other fungi. Many of them are human pathogens, causing diseases like athlete's foot, ring worm and histoplasmosis. The common genera are *Alternaria*, *Circospora*, *Fusarium*, etc.

**(e) Division Chytridiomycota:** The simplest of the true fungi belong to the division *Chytridiomycota*. This division contains one class, *Chytridiomycetes* and its members are known as the chytrids. These are simple terrestrial and aquatic fungi that reproduce asexually by forming motile zoospores with single, posterior, whiplash flagella. The entire organism is microscopic in size and may consist of a single cell, a small multinucleate mass, or a true mycelium. Usually chitin is the major constituent of chytrid cell walls. Chytrids are thought to have been derived from a protozoan ancestor having similar flagellation. They are likely to be ancestral to the remaining groups of true fungi. Some can grow saprophytically on dead organic matter and some others are parasites of algae.

**(f) Division Myxomycota (Acellular Slime Molds):** Under appropriate conditions plasmodial (acellular) slime molds exist as streaming masses of colourful protoplasm that creep along in an amoeboid fashion over moist, rotting logs, leaves and other organic matter. Feeding is by phagocytosis. Because this streaming mass lacks cell walls, it is called a plasmodium.

**(g) Division Acrasiomycota (Cellular Slime Molds):** The vegetative stage of cellular slime molds consists of individual amoeboid cells termed myxamoebae. The myxamoebae feed phagocytically on bacteria and yeasts. When food is plenty, they divide repeatedly by mitosis and cytokinesis, producing new daughter myxamoebae. As their food supply is exhausted they form a sluglike pseudoplasmodium.

**(h) Division Oomycota:** The oomycetes (egg fungi) or water molds resemble true fungi only in appearance, consisting of finely branched filaments called hyphae. However, oomycetes have cell walls of cellulose, whereas the walls of most fungi are made of chitin. Oomycetes are also unlike the true fungi in that they have tubular mitochondrial cristae. Water molds such as *Saprolegnia* and *Achlya* are saprophytes that grow as cottony masses on dead algae and small animals, mainly in freshwater environments. They are important decomposers in aquatic ecosystems. Some water molds are parasitic on the gills of fish. Commonly occurring genera are *Peronospora*, *Phytophthora* and *Plasmopora*.

## 2.6 THE WORLD OF ALGAE

Algae are most common to aquatic systems (fresh water, marine, or brackish water) in which they may be suspended (planktonic) or attached and living on the bottom (benthic). A few algae live at the water-atmosphere interface and are termed neustonic. Some algae grow on moist rocks, wood, trees and on the surface of moist soil. Algae also live as endosymbionts in various protozoa, mollusks, worms and corals. Several algae grow as endosymbionts within plants, some are attached to the surface of various structures and a few lead a parasitic existence (See Chapter 9 and 10). Algae also associate with fungi to form lichens.

The eucaryotic algal cell is surrounded by a thin, rigid cell wall. Some algae have an outer matrix lying outside the cell wall. This usually is flexible and gelatinous, similar to bacterial capsules. Very few algae have flagella, which are the locomotor organelles. There is a true nucleus, chloroplast with pyrenoids and other membranous organelles. Mitochondrial structure varies greatly in the algae. Some algae (euglenoids) have discoid cristae; some have lamellar cristae (green and red algae) and the remaining have tubular cristae (golden-brown and yellow-green, brown and diatoms).

Algae can be either autotrophic or heterotrophic. Most are photoautotrophic requiring only light and CO<sub>2</sub> as their principal source of energy and carbon. Chemoheterotrophic algae (many planktonic green algae and cyanobacteria) require external organic compounds as carbon and energy sources. There are striking variations in the structure of algal cells and thalli and some of the structural complexities are found typical to a definite group of algae. There are also remarkable complexity of multicellular forms, such as the giant kelps. Single-celled algae may be as small as bacteria or even believed to be smaller (nanoplankton and picoplankton of open oceans). Algae are unicellular, colonial, filamentous, membranous and blade like, or tubular (Fig.2.12).

**(a) Division Chlorophyta (Green Algae):** This is an extremely varied division. These algae grow in fresh and salt water, in soil and on and within other organisms (commensal, parasitic or symbiotic). The photosynthetic pigments are chlorophylls *a* and *b* along with specific carotenoids and they store carbohydrates as starch. Many have cell walls of cellulose. They exhibit a wide diversity of body forms, ranging from unicellular to colonial, filamentous, membranous or sheet-like and tubular types. Some species have a holdfast structure that anchors them to the substratum. Both asexual and sexual reproduction occur in green algae. Most common genera are *Chlamydomonas*, *Chlorella*, *Chlorococcum*, *Pandorina*, *Eudorina*, *Selenastrum*, *Ankistrodesmus* and *Volvox*. From *Chlamydomonas*, several distinct lines of evolutionary specialization have evolved in the green algae. The first line contains nonmotile unicellular green algae, such as *Chlorella*, *Chlorococcum*, *Selenastrum* and *Ankistrodesmus*. *Volvox* represent a second major line of evolutionary specialization.

**Table 2.12:** The five kingdom system of classification of algae with their approximate diversity in the environment.

<i>Kingdom</i>	<i>Division</i>	<i>Common name</i>	<i>Approximate number of species</i>
Protista	Chlorophyta	Green algae	7500
	Charophyta	Stoneworts	250
	Euglenophyta	Euglenoids	700
	Chrysophyta	Diatoms	6000
	Pyrrhophyta	Dinoflagellates	1100
Plantae	Phaeophyta	Brown algae	1500
	Rhodophyta	Red algae	3900

Source: Prescott *et al.* (2005).

**(b) Division Charophyta (Stoneworts/Brittleworts):** The stoneworts are abundant in fresh to brackish waters and have a worldwide distribution. Often they appear as a dense covering on the bottom of shallow ponds. Some species precipitate calcium and magnesium carbonate from the water to form a limestone covering. *Chara* and *Nitella* are the two widely distributed members of the division.

**(c) Division Euglenophyta (Euglenoids):** The euglenoids share with the *Chlorophyta* and *Charophyta* the presence of chlorophylls *a* and *b* in their chloroplasts. The primary storage product is paramylon (a polysaccharide composed of ( $\beta$ -1,3 linked glucose), which is unique to euglenoids. They occur in fresh, brackish and marine waters and on moist soils. They often form water blooms in ponds and cattle water tanks. The representative genera *Euglena* and *Phacus* are commonly found in waters with high organic load.

**(d) Division Chrysophyta (Golden-Brown and Yellow-Green Algae; Diatoms):** The division is quite diversified with respect to pigment composition, cell wall and type of flagellated cells. It is divided in three major classes: golden-brown algae, yellow-green algae and diatoms. The major



**(g) Division Rhodophyta (Red Algae):** It includes most of the seaweeds. A few members are unicellular but most are filamentous and multicellular. Some red algae are up to 1 m long. The stored food is the carbohydrate called floridean starch (composed of  $\alpha$ -1,4 and  $\alpha$ -1,6 linked glucose residues). Photosynthetic pigments are chlorophylls *a* and *d*, xanthophylls and phycobiliproteins. The characteristic colour is caused by the red pigment phycoerythrin. The presence of phycobiliproteins enables the members to live at depths of 100m or more. The cell walls of most red algae include a rigid inner part composed of microfibrils and a mucilaginous matrix. The matrix is composed of sulphated polymers of galactose called agar, funori, porphysan and carrageenan. Many red algae also deposit calcium carbonate in their cell wall and play an important role in building coral reefs. The common genera are *Batrachospermum*, *Porphyridium*, *Bangia*, *Lomentaria*, etc.

## 2.7 THE WORLD OF PROTOZOA

Protozoa are protists exhibiting heterotrophic nutrition and various types of locomotion. They occupy a vast array of habitats and niches and have organelles similar to those found in other eucaryotic cells and also specialized organelles. Moisture is absolutely necessary for the existence of protozoa because they are susceptible to desiccation. Most protozoa are free living and inhabit freshwater or marine environments. Many terrestrial protozoa can be found in decaying organic matter, in soil and even in beach sand; some are parasitic in plants or animals.

Protozoa play a significant role in the economy of nature. For example, they make up a large part of plankton that are an important link in many aquatic food chains and food webs of aquatic environments. They are also useful in biochemical and molecular biological studies. Some of the most important diseases of humans and animals are caused by protozoa (Table 2.13). Most protozoa are chemoheterotrophic.

**Table 2.13:** Pathogenic protozoa that cause major diseases of domestic animals.

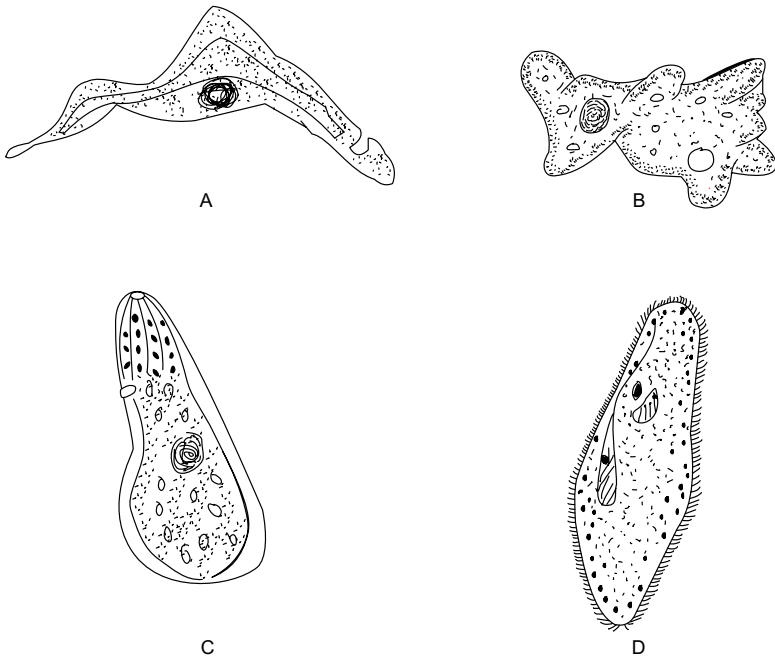
<i>Protozoan group</i>	<i>Genus</i>	<i>Host</i>	<i>Disease</i>
Amoebae	<i>Entamoeba</i>	Mammals	Amebiasis
	<i>Iodamoeba</i>	Pig	Enteritis
Sporozoa	<i>Babesia</i>	Cattle	Sarcocystis
	<i>Theileria</i>	Cattle, sheep	Theileriasis
	<i>Sarcocystis</i>	Mammals, birds	Sarcosporidiosis
	<i>Toxoplasma</i>	Cats	Toxoplasmosis
	<i>Isospora</i>	Dogs	Coccidiosis
	<i>Eimeria</i>	Cattle, cats, chickens, pig	Coccidiosis
	<i>Plasmodium</i>	Many animals	Malaria
	<i>Leucocytozoon</i>	Birds	Leucocytozoonosis
	<i>Cryptosporidium</i>	Mammals	Cryptosporidiosis

**Table 2.13:** (Contd.)

Ciliates Flagellates	<i>Balantidium</i>	Pig	Balantidiasis
	<i>Leishmania</i>	Dogs, horses, sheep, cattle	Leishmaniasis
	<i>Trypanosoma</i>	Most animals	Trypanosomiasis
	<i>Trichomonas</i>	Horses, cattle	Trichomoniasis
	<i>Histomonas</i>	Birds	Blackhead disease
	<i>Giardia</i>	Mammals	Giardiasis

### 2.7.1 Morphological Variations

Because protozoa are eucaryotic cells, in many respects their morphology and physiology are the same as the cells of multicellular animals (Fig. 2.13). However, because all of life's various functions must be performed within the individual protozoan, some morphological and physiological features are unique to protozoan cells. In some species the cytoplasm immediately under the plasma membrane is the semisolid or gelatinous ectoplasm, giving some rigidity to the cell body. The bases of the flagella or cilia and their associated fibrillar structures are embedded in the ectoplasm. The plasma membrane and structures immediately beneath it are called the pellicle. Inside the ectoplasm is endoplasm, which is more fluid and granular in composition and contains most of the organelles. Some protozoa have one nucleus, others have two or more identical nuclei. Still other protozoa have two distinct types of nuclei - a macronucleus and



**Figure 2.13** - Some members of protozoa. (A) *Trypanosoma* sp., (B) *Amoeba* sp., (C) *Apicomplexa* sp. and (D) *Paramecium* sp.

**Table 2.14:** (Contd.)

Phylum: <i>Ascetospora</i>	Spore with one or more spiroplasm; no polar capsules or filaments; parasitic in invertebrates	<i>Haplosporidium</i>
Phylum: <i>Myxozoa</i>	Spores of multicellular origin; one or many polar capsules; all parasitic, especially in fish	<i>Myxosoma</i>
Phylum: <i>Ciliophora</i>	Simple cilia or compound ciliary organelles in at least one stage in the life cycle; two types of nuclei; contractile vacuole present; binary fission transverse; sexuality involving conjugation; most species free living, but many commensal, some parasitic	<i>Balantidium</i> <i>Didinium</i> <i>Entodinium</i> <i>Ichthyophthirius</i> <i>Nyctotherus</i> <i>Paramecium</i>

Source: Prescott *et al.* (2005).

**(a) Phylum Sarcocystophora:** Protists that have a single type of nucleus and possess flagella or pseudopodia are included in this phylum. Both sexual and asexual reproductions are seen in this phylum. *Giardia*, *Leishmania*, *Trichomonas*, *Trichonympha* and *Trypanosoma* are the common genera. There are also many symbiotic amoebae in this phylum, most of which live in other animals. Two common genera are *Endamoeba* and *Entamoeba*.

**(b) Phylum Labyrinthomorpha:** It is a small phylum consisting of protists that have spindle-shaped or spherical nonamoeboid vegetative cells. In some genera, amoeboid cells move within a network of mucous tracks using a typical gliding motion. Most members are marine and either saprozoic or parasitic on algae. *Labyrinthula* is the most commonly occurring genus.

**(c) Phylum Apicomplexa:** The apicomplexans, often collectively called the sporozoans, have a spore-forming stage in their life cycle and lack special locomotory organelles. They are either intra- or intercellular parasites of animals and are distinguished by a unique arrangement of fibrils, microtubules, vacuoles and other organelles, collectively called the apical complex, which is located at one end of the cell. The four most important sporozoan parasites are *Plasmodium*, *Cryptosporidium*, *Toxoplasma* and *Eimeria*.

**(d) Phylum Microspora:** The small microsporans are obligatory intracellular parasites lacking mitochondria. Many of these protozoa are pathogenic. There has been an increased interest in these parasites because of their possible role as biological control agents for certain insects. Important genera are *Nosema*, *Encephalitozoon*, *Pleistophora*, *Microsporidium*, *Vittaforma*, *Trachipleistophora* and *Enterocytozoon*.

**(e) Phylum Ascetospora:** It is a relatively small phylum consisting exclusively of parasitic protists characterized by spores lacking polar caps or polar filaments. Ascetosporans, such as *Haplosporidium* are parasitic primarily in the cells, tissues and body cavities of mollusks.