Taxonomy, Physiology, and Ecology of Aquatic Microorganisms

4

Abstract

The principles behind the taxonomy of the microorganisms, especially the molecular approach (using the sequence of the 16S RNA in the small subunit of the ribosome) in the identification of bacteria, are discussed. The detailed taxonomy of bacteria, fungi, algae, protozoa, and viruses (including bacteriophages) is discussed, and emphasis is laid on those microorganisms which are aquatic. The chapter includes information on some of the smaller macroorganisms found in water such as nematodes and rotifers. The activities of aquatic microorganisms in photosynthesis, and the global cycling of nitrogen and sulfur is discussed.

Keywords

Taxonomy of microbial groups • Photosynthesis in aquatic microorganisms • Aquatic nitrogen and sulfur cycles • Rotifers and nematodes • Bacteria • Fungi • Protozoa • Algae • Three domains of living things • Woese • 16S or 18S RNA

4.1 Taxonomy of Microorganisms in Aquatic Environments

In this section, the classification of microorganisms will be discussed and emphasis will be laid on those microorganisms found in aquatic systems. The following are the organisms to be discussed:

Bacteria
Archeae
Eukarya
Fungi
  Algae
  Protozoa
Viruses

4.1.1 Nature of Modern Taxonomy

Modern taxonomy is the science of biological classification. It consists of three sections:

(a) *Classification*: The theory and process of arranging organisms into taxonomic groups or taxa (singular, taxon), on the basis of shared properties.
(b) *Nomenclature*: The assignment of names to taxonomic groups.
(c) *Identification*: The determination of the taxon to which a particular organism belongs, based on the properties of the organism.

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Taxonomy is important because it:

1. Allows the orderly organization of huge amounts of information regarding organisms
2. Enables predictions about their properties and formation of hypothesis about them
3. Facilitates the accurate characterization and identification of "unknown" organisms
4. Places organisms in meaningful manageable groups and thus facilitates scientific communication

4.1.2 Evolution of the Classification of Living Things

Landmarks in the evolution and development of biological classification may be ascribed to the contributions of the following:

1. *Linnaeus (1707–1778)*
   The Swedish naturalist, Carolus Linnaeus, is credited with introducing the earliest organized classification of living things in his *Systema Naturae* or natural system. He divided living things into plants and animals. Based on morphology and motility, the distinction between the two groups of organisms was clear: plants were green and did not move; on the other hand, animals were not green, but moved.
2. *Ernst Haeckel (1834–1919)*
   Soon after the discovery of the microscope, previously invisible microscopic organisms were observed, some of which had properties common to both plants and animals. Some such as *Euglena* were green like, but they also moved about like animals. Because the clear-cut criteria which separated plants from animals were absent in these "new" organisms, the German biologist who was a contemporary of Charles Darwin, in 1866 coined the name Protista for a third kingdom, in addition to the Plant and Animal Kingdoms.
3. *Robert Harding Whittaker (1920–1980)*
   Whittaker was an American. Born in Wichita, Kansas, he worked in various places including the University of California, Irvine, and Cornell University. In 1968, he proposed the five-kingdom taxonomic classification of living things into the Animalia, Plantae, Fungi, Protista (Algae and Protozoa), and Monera (Bacteria). His categorization of living things was based on three criteria: cell-type (whether prokaryotic or eukaryotic); organizational level (unicellular or multi-cellular); nutritional type (autotrophy or heterotrophy).
4. *Carl R. Woese (1928–)*
   The current classification of living things is based on the work of Carl Robert Woese of the University of Illinois. While earlier classifications were based mainly on morphological characteristics and cell-type, following our greater understanding of living things at the molecular level, Woese's classification is based on the sequence of the gene of the ribosomal RNA (rRNA) in the 16S of the small subunit of the prokaryotic ribosome, or the 18S of the small subunit of the eukaryotic ribosome (Petti et al. 2006).

The sequence of the rRNA in the 16S or 18S of the small subunit of the ribosome is used for the following reasons:

(a) The ribosome is an important organelle in all living things where it is used for a basic function for the support of life, namely, protein synthesis.
(b) The 16S (prokaryote) or 18 S (eukaryote) rRNA is an essential component of the ribosome.
(c) The function of 16S or 18 S rRNA is identical in all ribosomes.
(d) The sequences of the 16S or 18 S rRNA are ancient (or highly conserved) and change only slowly with evolutionary time.
(e) Organisms can generally inherit genes in two ways: From parent to offspring (vertical gene transfer), or by horizontal or lateral gene transfer, in which genes jump between unrelated organisms, a common phenomenon in prokaryotes. There is little or no lateral gene transfer in the sequences in the 16S or 18 S RNA of the ribosomal small units.

All the above properties make the sequence of the rRNA in the 16S or 18S of the small subunit of the ribosome useful as molecular chronometers for measuring evolutionary changes among organisms. Using this method, living things are now divided into three domains: Archae, Bacteria, and Eukarya. A diagrammatic representation of the three domains is given in Fig. 4.1, and their distinguishing properties are given in Table 4.1 (Woese 1987, 2000, 2002).

4.1.3 Determining Taxonomic Groups Within Domains

The smallest unit of biological classification is the *Species*. Species sharing similar properties are put in a *Genus*. Genera (plural of genus) sharing similar characteristics are put in a *Family*. Families with similar properties are arranged in an *Order*. Orders with similar properties are classified as into a *Class*. Classes which share similar properties are grouped into a *Phylum*. Phyla (plural of phylum) with similar properties are put in a *Kingdom* and similar kingdoms are in a *Domain*.

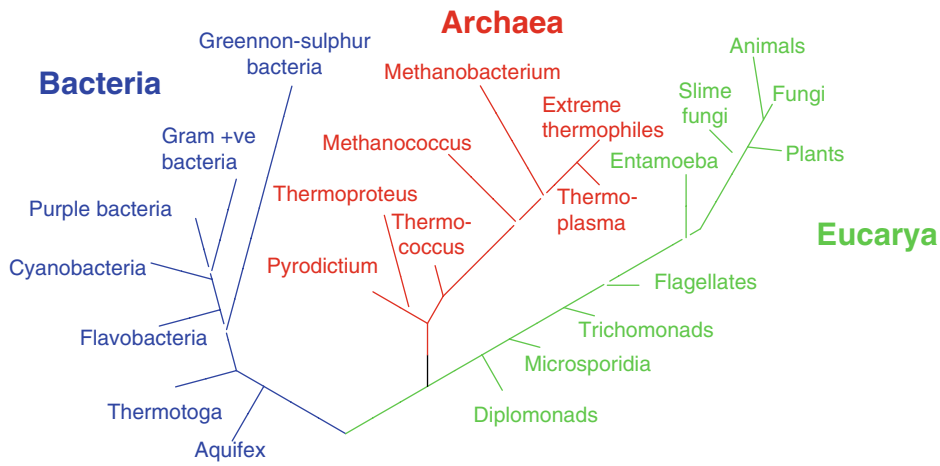


Fig. 4.1 Three domains of living things based on Woese's work (Modified from Ciccarelli et al. 2006)

Table 4.1 Summary of differences among the three domains of living things (Modified from Madigan and Martinko 2006)

S. No.	Characteristic	Bacteria	Archae	Eukarya
1.	Prokaryotic cell structure	+	+	–
2.	DNA present in closed circular form	+	+	–
3.	Histone proteins present[a]	–	+	+
4.	Nuclear membrane	–	–	+
5.	Muramic acid in cell wall	+	–	–
6.	Membrane lipids: Fatty acids or branched hydrocarbons	Fatty acids	Branched hydrocarbons	Fatty acids
	Unbranched fatty acid chains attached to glycerol by ester linkages	+	–	+
7.	Ribosome size	70 S	70 S	80 S
8.	Initiator t-RNA	Formyl-methionine	Methionine	Methionine
9.	Introns in most genes[b]	–	–	+
10.	Operons[c]	+	+	–
11.	Plasmids	+	+	Rare
12.	Ribosome sensitive to diphtheria toxin	–	+	+
13.	Sensitivity to streptomycin, chloramphenicol, and kanamycin	+	–	–
Physiological/special structures				
14.	Methanogenesis	+	+	–
15.	Denitrification	+	+	–
16.	Nitrogen fixation	+	+	–
17.	Chlorophyll-based photosynthesis	+	–	+ (Plants)
18.	Gas vesicles	+	+	–
19.	Chemolithotrophy	+	+	–
20.	Storage granules of poly-β-hydroxyalkanoates	+	+	–
21.	Growth above 80°C	+	+	–
22.	Growth above 100°C	–	+	–

[a]Histone proteins are present in eukaryotic chromosomes; histones and DNA give structure to chromosomes in eukaryotes; proteins in archeae chromosomes are different
[b]Noncoding sequences within genes
[c]Operons: Typically present in prokaryotes, these are clusters of genes controlled by a single operator
[d]TATA box (also called Hogness Box): an AT-rich region of the DNA with the sequence TATAT/AAT/A located before the initiation site
[e]Transcription factor is a protein that binds DNA at a specific promoter or enhancer region or site, where it regulates transcription

Several mnemonics exist to help with remembering the correct order of the listing of taxonomic groups. Two are given below:

Domain	**K**ingdom	**P**hylum	**C**lass	**O**rder	**F**amily	**G**enus	**S**pecies
David	**K**indly	**P**ay	**C**ash	**O**r	**F**urnish	**G**ood	**S**ecurity
Dignified	**K**ings	**P**lay	**C**hess	**O**n	**F**ine	**G**reen	**S**ilk

The names of the ascending taxonomic groups to which the fruit fly, humans, peas, and the bacterium, *E coli* belong are given in Table 4.2.

4.1.3.1 Definition of Species

In the Domain Eukarya, a species is defined as a group of organisms which can mate and produce fertile offspring. Even though goats are of different kinds, they will mate and produce fertile offspring; similarly with dogs. The horse and the donkey are of different species, because although they can mate and produce offspring, the offspring are not fertile. This definition is occasionally complicated by the lateral transfer of genes.

In Bacteria and Archae, the definition is a little different. A species in these Domains is a collection of strains that share many stable properties and differ significantly from other groups.

4.1.3.2 Nomenclature of Biological Objects

Biological objects, including microorganisms are named in the binomial system devised by Carolus Linnaeus. The genus name is written first and begins with an uppercase (capital) letter; the other half of the name is written in lowercase (small) letters and is the species name. The two are written in italics or underlined if written in long hand. When written formally, the name of the author who first described the organism is included and the year of the publication is given; the names are usually written in Latin or latinized. The name of a hypothetical Bacillus discovered in water by John Smith and published in 2007 could be *Bacillus aquanensis* Smith 2007. Usually only the genus and species names are given; the author and year of publication are omitted (van Regenmortel 1999).

4.1.3.3 Criteria and Methods for the Identification and Classification of Bacteria and Archae: Morphological, Physiological, Nucleic Acid, and Chemical Properties

Whereas members of the Domain Eukarya are classified largely on their morphological characteristics which are adequately diverse, morphological types are very limited in the Domains Bacteria and Archae. Therefore, while morphological properties are used, other characteristics are employed in addition to morphology. The properties used for classifying and identifying unknowns among organisms in the Domains Bacteria and Archae are given in Table 4.3. The principles of methods used are described briefly below.

Morphological and Physiological Methods

1. *Nutritional types of Bacteria*

   Living things are classified into major nutritional types on the basis of the following attributes:

   (a) Carbon source utilized

   A carbon skeleton is required for the compounds used for growth and development such as carbohydrates, amino acids, fats, etc. The organism is *autotrophic* if it manufactures its food and obtains its carbon through fixing CO_2 such as is the case with plants, algae, and some bacteria. When the organism cannot manufacture its own food from CO_2 but must utilize food already manufactured from CO_2, in the form of carbohydrates, proteins etc., it is *heterotrophic*. This is the case with animals and most bacteria.

Table 4.2 Taxonomic groups of some organisms (From Anonymous 2008a)

Rank	Human	Fruit fly	Pea	*E. coli*
Domain	Eukarya	Eukarya	Eukarya	Bacteria
Kingdom	Animalia	Animalia	Plantae	Bacteria
Phylum (animals) or division (plants)	Chordata	Arthropoda	Magnoliophyta	Proteobacteria
Class	Mammalia	Insecta	Magnoliopsida	Gammaproteobacteria
Order	Primates	Diptera	Fabales	Enterobacteriales
Family	Hominidae	Drosophilidae	Fabaceae	Enterobacteriaceae
Genus	*Homo*	*Drosophila*	*Pisum*	*Escherichia*
Species	*H. sapiens*	*D. melanogaster*	*P. sativum*	*E. coli*

Table 4.3 Some properties used for bacterial classification and identification

S. No.	Property
1.	Nutritional type
	(i) Autotrophy
	(ii) Heterotrophy
2.	Energy release
	(i) Lithotrophy
	(ii) Organotrophy
3.	Cell wall: Gram reaction
	(i) Gram negative
	(ii) Gram positive
4.	Cell morphology
	(i) Cell shapes
	(ii) Cell aggregation
	(iii) Flagellation – motility
	(iv) Spore formation and location
	(v) Special staining, e.g., Ziehl–Nielsen
5.	Physiological properties
	(i) Utilization of various sugars
	(ii) Utilization of various polysaccharides
	(iii) Utilization of various nitrogenous substrates
	(iv) Oxygen requirement
	(v) Temperature requirements
	(vi) pH requirement
	(vii) Production of special enzymes, e.g., catalase, coagulase, optochin, oxidase
6.	Antigenic properties
7.	Molecular (nucleic acid) methods
	(i) G + C composition
	(ii) DNA:DNA hybridization
	(iii) Ribotyping
	(iv) Fluorescent in-situ hybridization (FISH)
8.	Chemical analysis (Chemotaxonomy)
	(i) Lipid analysis
	(ii) Protein analysis

(b) Source of reducing equivalent
During the generation of energy in the cell, electrons are transferred from one compound to another. An organism is said to be *organotrophic* when it uses organic compounds as a source of electrons. When the source of electrons is inorganic, it is said to be *lithotrophic*.

(c) Source of energy
Some organisms derive energy for the generation of ATP used for the biosynthesis of new compounds and other cellular activities from sunlight; such organisms are *phototrophic*. When the generation of ATP occurs through energy obtained from chemical reactions, the organism is said to be *chemotrophic*.

The carbon source utilized, the source of reducing equivalent, and the source of energy determine the nutritional type of bacteria, and a wide variety of combinations of these three is possible. Table 4.4 gives a selection of the possible permutations.

2. *Cell wall: Gram reaction*
The Gram stain was devised by the German doctor, Christian Gram in 1884 and divides bacteria into two groups: Gram positive and Gram negative. On account of the greater thickness of peptidoglycan in the Gram positive wall (see Fig. 4.2), the iodine-crystal violet stain in the Gram stain is retained when decolorized with dilute acid, whereas it is removed in the Gram negative cell wall. The Gram stain also divides all bacteria into two groups regarding their susceptibility to the classical antibiotic penicillin: Gram-positive bacteria, being susceptible, while Gram negative bacteria are not (Fig. 4.3).
3. *Cell morphology*
(a) Individual cell shapes
Cell shapes in bacteria are limited and are spheres (coccus– cocci, plural), rods, spiral, or comma or vibrio (see Fig. 4.4).

Table 4.4 Nutritional types of living things

S/No	Nutritional type	Energy source	Carbon source	Reducing equivalent	Example
1	Photoautotrophs	Light	CO_2	Organotrophic	Plants, Cyanobacteria
2	Photoautotrophs	Light	CO_2	Lithotrophic $H_2S \rightarrow S$	Sulfur bacteria e.g., *Beggitoa* sp.
3	Chemoautotrophs	Chemotrophic	CO_2	Oxidation of sulfur	*Thiobacillus oxidans*
4	Photoheterotrophs	Light	Organic compounds	Organotrophic	Purple non-sulfur bacteria
5	Chemoheterotrophs	Chemotrophic	Organic compounds	Organotrophic	Animals, fungi, protozoa, most bacteria

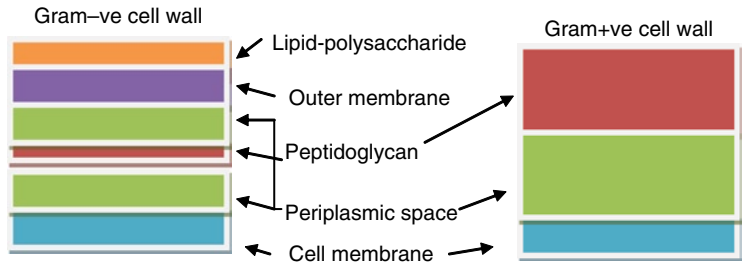


Fig. 4.2 Diagram illustrating the generalized structure of the bacterial cell wall (Note the comparative thicknesses of the peptidoglycan layers in the Gm+ve and Gm−ve walls)

Note that the peptidoglycan layer is very thick in Gram +ve walls, but very thin in Gram –ve bacterial cells. This thick peptidoglycan enables Gram +ve walls retain crystal violet, the primary stain in the Gram, when decolorized with dilute acid. Crystal violet is not retained in Gram –ve bacteria because the peptidoglycan layer is thin. The Gram –ve wall would be colorless after decolorization with dilute acid. However in the Gram stain, after decolourization, the cells are counterstained with a red stain, safranin. On account of this the Gram negative cells appear red in the Gram stain, while Gram +ve cells are violet (see text and Fig. 4.3)

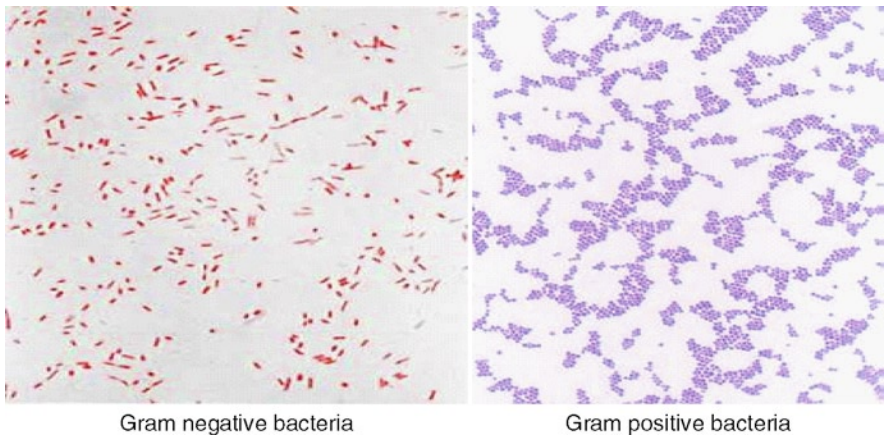


Fig. 4.3 Gram staining

(b) Cell aggregates
Cocci can occur in pairs (diplococci), in chains (streptococci), or in clumps (staphylococci). Rods may occur in short chains of two or three or in long chains or filaments.

(c) Flagellation
The flagella may be at one end (polar) and may occur singly or as a tuft. The flagella may occur all around the cell when it is peritrichous (see Fig. 4.5).

(d) Spores and location of spores
Spores are bodies resistant to heat and other adverse conditions which may be terminal or placed mid-way in the cell; in either position, it may be less than the diameter of the cell or may be wider. The terminal wider spore gives the shape of a drumstick, and is diagnostic of the anaerobic rod-like spore-former, *Clostridium tetanii*, the causative agent of tetanus (see Fig. 4.6).

(e) Acid-fast (Ziel–Nieelsen) stain
If the bacterium is suspected to belong to *Mycobacterium* spp., or any of the other acid-fast bacteria it might be stained with hot basic fuchsin; acid fast bacteria retain the dye when decolorized.

4. *Utilization of various substrates*
Utilization of various sugars, carbohydrates, and nitrogenous sources

The ability of the organism to produce acid and/or gas from a medium containing a particular substrate is diagnostic of its ability to utilize it. The utilization of a wide range of sugars and other carbohydrates, and nitrogen sources including urea is tested by the presence of gas in the small (Durham) tube placed in the

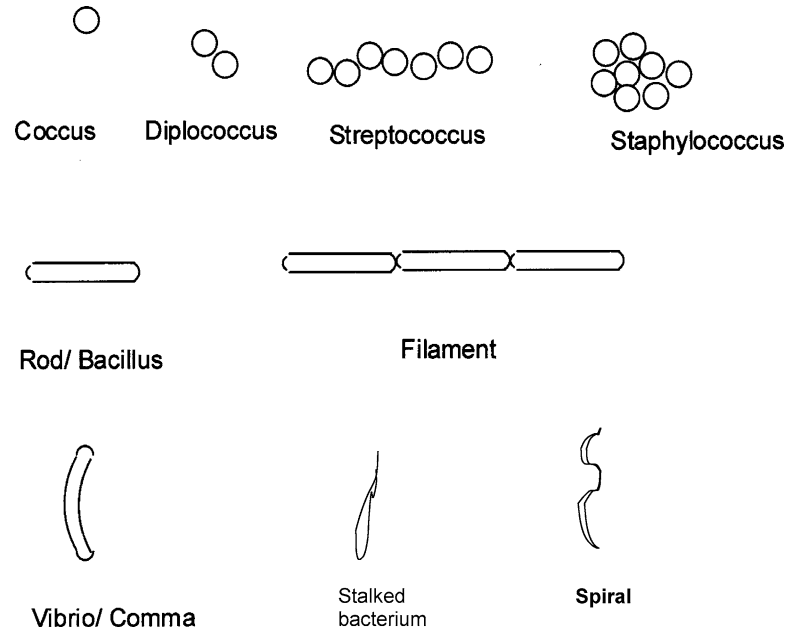


Fig. 4.4 Bacterial cell shapes

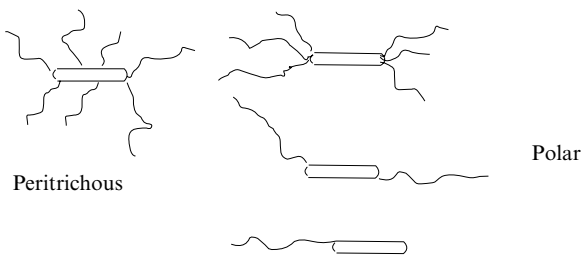


Fig. 4.5 Bacterial flagellation

Fig. 4.6 Spore locations in the bacterial cell

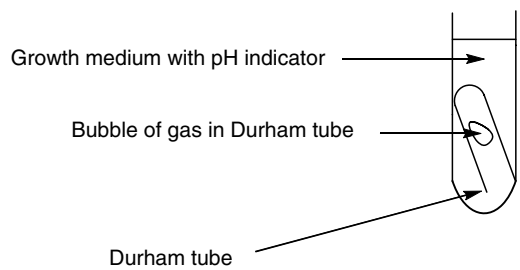


Fig. 4.7 Setup for testing bacterial utilization of various substrates

test tube containing the medium; a change in the color of indicator would indicate acid production by the organism (see Fig. 4.7).

5. *Determination of optimum growth conditions*
   Optimum pH, temperature, and oxygen requirements are determined by growing the organism under different conditions of pH and temperature and finding the best condition. For oxygen requirement, the organism may be grown in an agar stab and sealed with sterile molten petroleum jelly to determine if it will grow under anaerobic conditions.
6. *Secretion of special enzymes*
   The secretion of unique enzymes is diagnostic. Some of the following enzymes are diagnostic (see Fig. 4.7a).

Coagulase: Coagulase is an enzyme produced by *Staphylococcus aureus* that converts fibrinogen to fibrin. In the laboratory, it is used to distinguish between different types of *Staphylococcus* isolates. Coagulase negativity excludes *S. aureus*. The coagulase test is used to differentiate *Staphylococcus aureus* from the other species of *Staphylococcus*. The test uses rabbit plasma that has been inoculated with a staphylococcal colony. The tube is then incubated at 37°C for about 90 min. If positive (i.e., the suspect colony is *S. aureus*), the serum will coagulate, resulting in a clot. If negative (i.e., if the tested colony is *S. epidermidis*), the plasma remains liquid.
Catalase: Catalase is a common enzyme found in living organisms. Its functions include catalyzing the decomposition of hydrogen peroxide to water and oxygen.

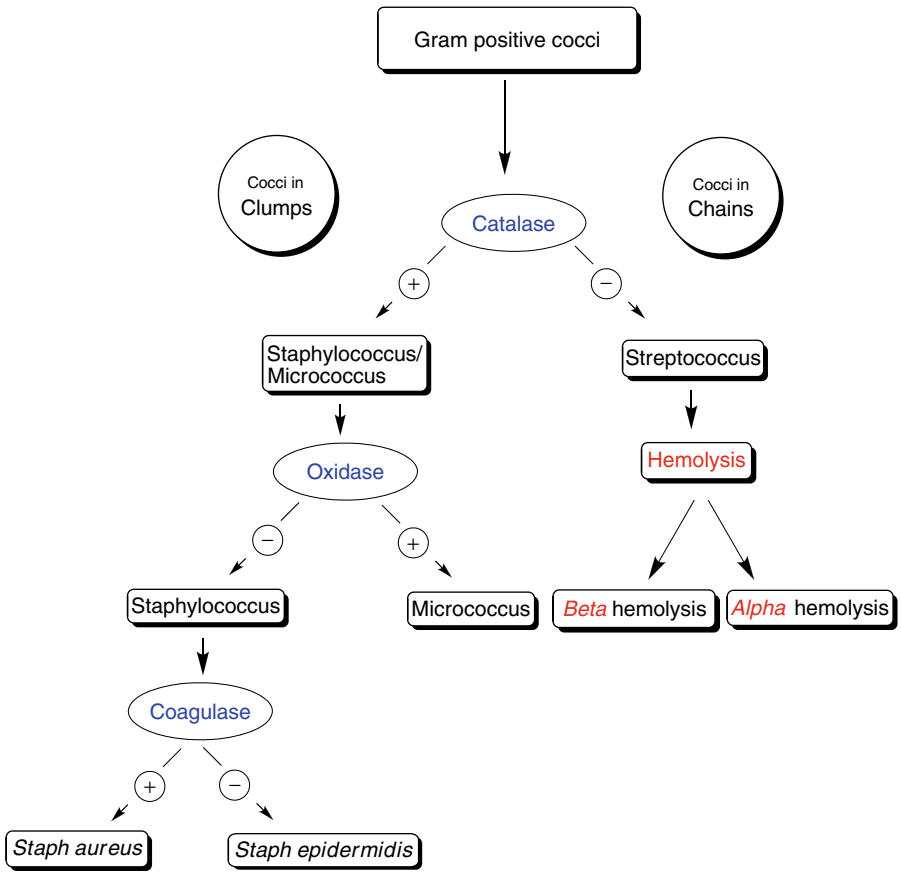


Fig. 4.7a The use of Special Enzymes secreted by some Gram positive cocci in their Classification

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

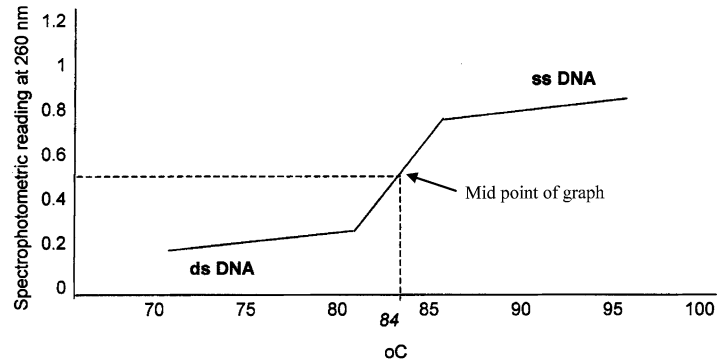
In microbiology, the *catalase test* is used to differentiate between staphylococci and micrococci, which are catalase-positive, from streptococci and enterococci, which are catalase-negative

Optochin: Optochin (ethyl hydrocuprein hydrochloride) is used for the presumptive identification of *Streptococcus pneumoniae*, which is optochin sensitive, from *Streptococcus viridans* which is resistant. Bacteria that are optochin sensitive will not continue to grow (i.e., *Streptococcus pneumoniae* will die), while bacteria that are not optochin sensitive will be unaffected (i.e., *Streptococcus viridans* will survive).

Oxidase: An oxidase is any enzyme that catalyzes an oxidation/reduction reaction involving molecular oxygen (O_2) as the electron acceptor. In these reactions, oxygen is reduced to water (H_2O) or hydrogen peroxide (H_2O_2). The oxidases are a subclass of the oxidoreductases. In microbiology, the oxidase test is used as a phenotypic character for the identification of bacterial strains; it determines whether a given bacterium produces cytochrome oxidases (and therefore utilizes oxygen with an electron transfer chain) (see Fig. 4.7a).

7. *Serology*

Bacterial species and serotypes can be identified by specific antigen/antibody reactions. Antigens are substances that induce the production of antibodies in an animal body. Bacteria and bacterial components



ss DNA = Single stranded DNA
ds DNA= Double stranded DNA

Fig. 4.8 Determination of temperature of melting (Tm) of DNA

serve as excellent antigens. The test includes production of antibodies in an animal host and testing of the antiserum by either the agglutination or precipitation test. In the agglutination test, a drop of the culture of a particular bacterium is mixed on a slide with the anti-serum of an individual infected by it and examined under a microscope. If clumping occurs, the test bacterium is considered to be the same or closely related to the bacterium used as the antigen.

Nucleic Acid Methods

The methods for the characterization and identification of bacteria which have been discussed so far are based on phenotypic properties, i.e., the outward manifestation of the innate (genetic) attributes of the organism. The properties to be discussed in this section are those of the nucleic acids of the organisms. They alone however do not define the organism and must be taken along with the phenotypic properties. They are very useful in refining the description of an organism. They are particularly useful in identifying strains within a species.

(a) *G + C ratio*

The G + C ratio is the percentage of guanine + cytosine in an organism's DNA. Several methods exist for determining this ratio. One method is to determine the Tm or temperature of melting of the DNA.

At room temperature, DNA is double stranded. However, as its temperature is raised gradually, the two strands separate and the rapidity of separation with increasing temperature depends on the amount of G and C in the organism's DNA. G and C are linked by triple bonds and are therefore less likely to separate than A and T bonds, which have double bonds. The higher the G + C content, the higher the temperature at which the DNA separates completely.

DNA begins to separate at 70–75°C and separates completely at about 90°C, when it is said to have melted. When cooled slowly, it begins to anneal (i.e., to reform itself into double strands). In annealing, the strands do not return to their previous "partners" but will anneal with any strand with complimentary bases no matter the source, including those coming from the same organism, other organisms, or even those synthesized in the laboratory. This phenomenon of annealing with complimentary strands from any source is important in other procedures such as in the identification of unknowns, the Polymerase Chain Reaction (PCR), etc.

When the Tm method is used to determine the G + C composition, the temperature of the double (ds) DNA is raised slowly and subjected to spectrophotometric reading at 260 nm. The graph of the spectrophotometric readings is plotted against the change in temperature (see Fig. 4.8). The Tm is the midpoint of the resulting graph. Two organisms with similar phenotypic properties and the same G + C ratio are likely to belong to the species.

(b) *DNA–DNA hybridization*

This technique measures the degree of genetic similarity between pools of DNA sequences. It is usually used to determine the genetic distance between two species.

It was seen above that when melted DNA is allowed to cool slowly, the single-stranded DNA will anneal with any single-stranded DNA no matter its source, as long the bases are complimentary. To determine how closely related an unknown organism is with a known one, DNA from the two

Table 4.5 Some signature sequences unique to the domains (Modified from Madigan and Martinko 2006)

S/No	Oligonucleotide sequence	Occurrence in (%)		
		Archaea	Bacteria	Eukarya
1	CACACCCG	100	0	0
2	CAACCYCR	0	>95	0
3	UCCCUG	>95	0	100
4	UACACACCG	0	>99	100

Y = Any pyridine
R = Any purine

organisms are mixed and heated slowly and allowed to anneal slowly. The unknown or the known is labeled with a fluorescent dye or with radioactive phosphorous and measured at 260 nm in the spectrophotometer. The extent of the taxonomic relatedness is reflected in the extent of the annealing. If the two organisms are of the same species, there will be complete annealment. Some authors have suggested that organisms of the same species will have 90% annealing, while those of the same genus will have about 75% annealing.

(c) *Ribotyping*

Ribotyping is an RNA-based molecular characterization of bacteria. In ribotyping, bacteria genomic DNAs are digested and separated by gel electrophoresis. Universal probes that target specific conserved domains of ribosomal RNA coding sequences are used to detect the band patterns. Ribosomal genes are known to be highly conserved in microbes, meaning that the genetic information coding for rRNA will vary much less within bacteria of the same strain than it will between bacterial strains. This characteristic allows for a greater ability to distinguish between different bacterial strains.

In ribotyping, restriction enzymes (i.e., enzymes which cut DNA at specific positions) are used to cut the genes coding for rRNA into pieces, and gel electrophoresis is used to separate the pieces by size. Genetic probes then visualize locations of different-size fragments of DNA in the gel, which appear as bands. The banding pattern of DNA fragments corresponding to the relevant rRNA is known as the ribotype.

A probe is a strand of nucleic acid which is synthesized in the laboratory and can be labeled with a dye or radioactively. Probes are used to hybridize to a complementary nucleic acid from a mixture. Probes can be general or specific. Thus, it is possible to design probes which will bind to sequences in the ribosomal RNA of all organisms irrespective of Domain. On the other hand, specific probes can be designed which will react only with nucleic acid of Bacteria, Archae, or Eukarya because of the unique sequences found in these groups. Even within species in the various domains, signature sequences exist which will enable the identification of the species using probes (see Table 4.5). Ribotyping is so specific that it has been nicknamed "molecular finger printing."

(d) *FISH – Fluorescent In Situ Hybridization*

This is a special type of ribotyping. In FISH, the whole organism is used without need to isolate the organism's DNA. The cells are treated with chemicals which make the cell walls and cell membranes permeable, thus permitting the entry of probes labeled with fluorescent dyes. After hybridization of the ribosomes with the dye, the entire organism fluoresces and can be seen under the light microscope. FISH is widely used in ecological and clinical studies. It can be used for the rapid identification of bacterial pathogens in clinical specimens; ordinary procedures take about 48 h, but FISH can be completed in a few hours.

Chemical Analysis of Microbial Components for Taxonomic Purposes (Chemotaxonomy)

(a) *Protein analyses*

Proteins are isolated from the whole bacterium, the cell membrane, or the ribosome. The proteins are run in a two-dimensional gel electrophoresis on polyacrylamide gel. The first run separates the proteins on the basis of their molecular weights and the second on the basis of their iso-electric points (Ochiai and Kawamoto 1995). The resulting protein pattern is diagnostic of a particular organism. If many samples are examined, the

Table 4.6 Principal gas chromatography (GC) fatty acid methyl ester (FAME) products of *B. pseudomallei* and *B. thailandensis* (From Inglis et al. 2003. With permission)

	% of total FAME content for:	
FAME peak	*B. pseudomallei* (n = 87)	*B. thailandensis* (n = 13)
18:1 w7c	32	32
16:0	23	25
17:0 cyclo	5.7	5.5
16:0 3OH	4.1	4.6
19:0 cyclo w8c	3.7	3.8
14:0	3.5	2.6
18:0	0.9	1.1
14:0 2OH[a]	0.58	Not detected

[a]http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=254375&rendertype=table&id=t1#t1fn3

patterns can be scanned with a computer. Patterns from unknown organisms are compared with patterns of known organisms to determine the relatedness of the known to the unknown.

(b) *Fatty acid analyses – fatty acid methyl ester (FAME)*

This method is widely used in clinical, food, and water microbiology for the identification of bacteria. Fatty acids from the cell membrane of bacteria as well from the outer membrane of Gram negative bacteria are extracted and converted to their methyl esters. The esters are then run in a gas chromatograph. The patterns of the gas chromatograms are diagnostic and can be used to identify unknowns. For example, *Burkholderia pseudomallei*, the cause of melioidosis, has been distinguished from the closely related but non-pathogenic *Burkholderia thailandensis* by gas chromatography (GC) analysis of fatty acid derivatives. A 2-hydroxymyristic acid derivative (14:0 2OH) was present in 95% of *B. pseudomallei* isolates but absent from all *B. thailandensis* isolates (see Table 4.6) (Inglis et al. 2003; Banowetz et al. 2006).

4.1.4 Bacteria

4.1.4.1 Taxonomic Groups Among Bacteria

Bacterial groups are described in two compendia, *Bergey's Manual of Determinative Bacteriology* and *Bergey's Manual of Systematic Bacteriology.* The first manual (on *Determinative Bacteriology*) is designed to facilitate the identification of a bacterium whose identity is unknown. It was first published in 1923 and the current edition, published in 1994, is the ninth. The companion volume (on *Systematic Bacteriology*) records the accepted published descriptions of bacteria, and classifies them into taxonomic groups. The first edition was produced in four volumes and published between 1984 and 1989. The bacterial classification in the latest (second) edition of *Bergey's Manual of Sytematic Bacteriology* is based on 16S RNA sequences, following the work of Carl Woese, and organizes the Domain Bacteria into 18 groups (or *phyla*; singular, *phylum*). It is to be published in five volumes: Volume 1 which deals with the *Archae* and the deeply branching and phototrophic bacteria was published in 2001; Volume 2 published in 2005 deals with the *Proteobacteria* and has three parts; Volume 3 (2009) and Volume 4 (2009) will deal with Firmicutes and The *Bacteroidetes*, *Planctomycetes*, *Chlamydiae*, etc. respectively; Volume 5 will be published in 2010 and deals with the *Actinobacteria* (Bergey's Manual Trust 2009; Garrity 2001–2006).

The manuals are named after Dr. D H Bergey who was the first Chairman of the Board set up by the then Society of American Bacteriologists (now American Society for Microbiology) to publish the books. The publication of *Bergey Manuals* is now managed by the Bergey's Manual Trust. Of the 18 phyla in the bacteria (see Figs. 4.9 and 4.10), the Aquiflex is evolutionarily the most primitive, while the most advanced is the Proteobacteria. In the following discussion, emphasis will be laid on the bacteria which are aquatic.

1. *Aquifex*

The two species generally classified in *Aquifex* are *A. pyrophilus* and *A. aeolicus*. Both are highly thermophilic, growing best in water temperature of 85–95°C. They are among the most thermophilic bacteria known. They can grow on hydrogen, oxygen, carbon dioxide, SO_2, S_2O_3, or NO_3 and mineral salts, functioning as a chemolithoautotroph (an organism which uses an inorganic carbon source for biosynthesis and an inorganic chemical energy source). As a hyperthermophilic bacterium, *Aquifex aeolicus* grows in extremely hot tempuratures such as near volcanoes or hot springs. They grow optimally at temperatures around 85°C but can grow at temperatures up to 95°C. It needs oxygen to carry on its metabolic machinery, but it can function in relatively low levels of oxygen. The genus *Aquifex* consists of Gram negative rods.

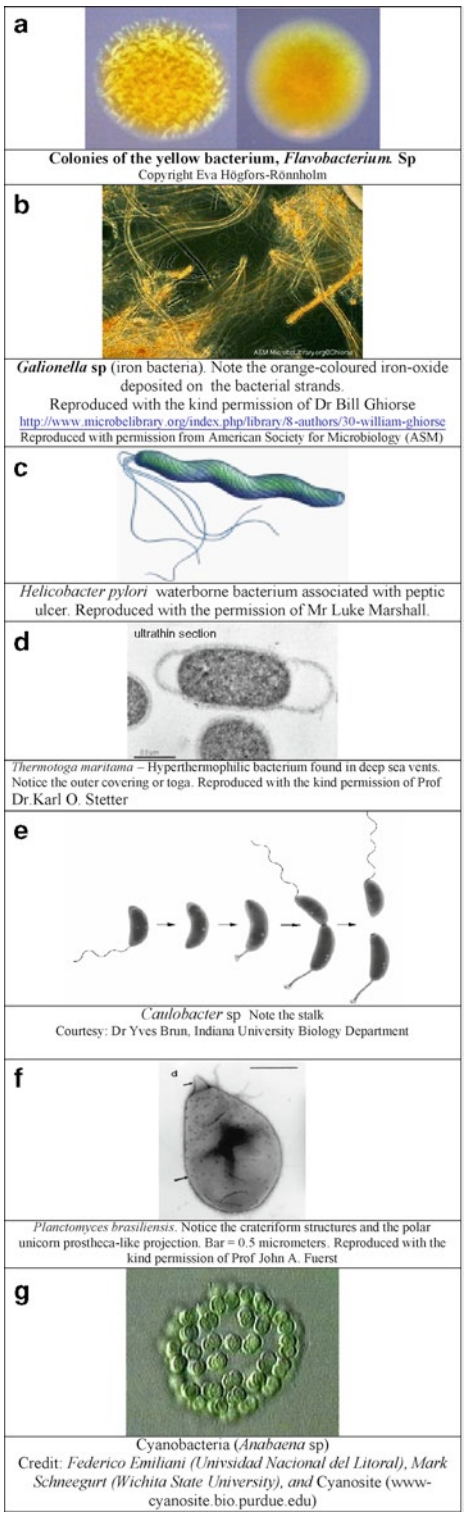


Fig. 4.9 Illustrations of some bacteria (All items in table reproduced with permission)

2. *Thermodesulfobacterium*
   Thermodesulfobacterium is a thermophilic sulfate reducer. Sulfate reducers include a wide range of morphological types, including rods, vibrios, ovals, spheres, and even tear-dropped or onion-shaped cells. Some are motile, others are not. Most sulfate-reducing bacteria are mesophilic, but a few are thermophiles, among which is the Gram negative and anaerobic *Thermodesulfobacterium*. The bacterium is non-spore-forming. It is an aquatic organism and has been isolated from volcanic hot springs, deep-sea hydrothermal sulfides, and other marine environments. In marine sediments and in aerobic wastewater treatment systems, sulfate reduction accounts for up to 50% of the mineralization of organic matter. Furthermore, sulfate reduction strongly stimulates microbially enhanced corrosion of metals. Sulfate Reducing Bacteria (SRB) are discussed in more detail later.
3. *Thermotoga*
   Thermotoga is typically a rod-shaped cell enveloped in an outer cell membrane (the "toga" or jacket). *Thermotoga* enzymes are known for being active at high temperatures. Enzymes from *Thermotoga* spp. are extremely thermostable and therefore, useful for many industrial processes such as in the chemical and food industries. The organisms are thermophilic or hyperthermophilic, growing best around 80°C and in the neutral pH range. The salt tolerance of *Thermotoga* species varies greatly; while some display an extremely high salt tolerance, others are restricted to low-salinity habitats. This aerobic Gram-negative organism is typically non-spore-forming and metabolizes several carbohydrates, both simple and complex, including glucose, sucrose, starch, cellulose, and xylan. It can grow by anaerobic respiration using H_2 as electron donor and Fe^{3+} as electron acceptor. It is found in hot springs and in the hydrothermic vents of ocean floors. *Thermotoga maritime* has been widely studied.
4. *Green non-sulfur bacteria (Chloroflexi)*
   The Green non-sulfur bacteria are now known as Chloroflexi are typically filamentous, and can move about by bacterial gliding. They are facultatively aerobic and have a different method of carbon fixation (photoheterotrophy) from other photosynthetic bacteria. Like green plants, they also carry out pho-

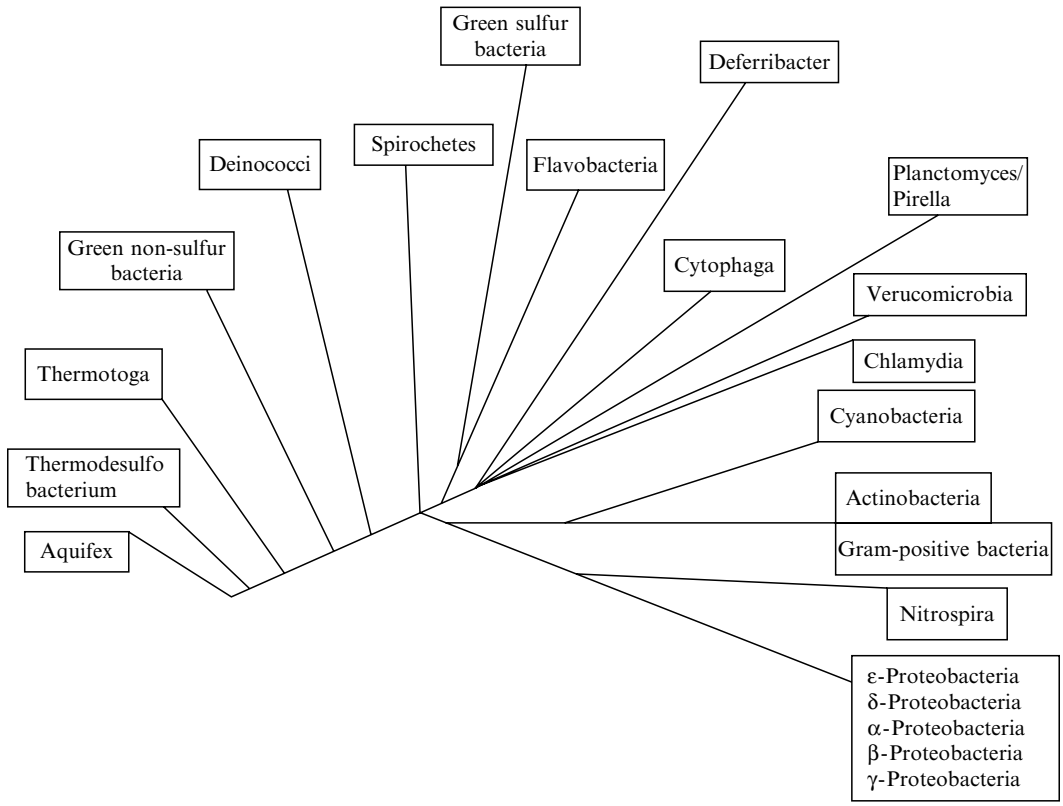


Fig. 4.10 Bacterial groups according to the 16S RNA classification

tosynthesis, but there are differences between the two; for instance, unlike plants, they do not produce oxygen during photosynthesis. The process of photosynthesis in the bacteria and in higher plants is discussed more fully below.

5. *Deinecoccus-Thermus*
   The *Deinococcus-Thermus* are a small group of Gram negative bacteria comprised of cocci which are highly resistant to environmental hazards because they are able to quickly repair damage to their DNA. There are two main groups. The Deinococcales include a single genus, *Deinococcus*, with several species that are resistant to radiation; they have become famous for their ability to "eat" nuclear waste and other toxic materials, survive in the vacuum of atmosphere space, and survive extremes of heat and cold. *Thermus* spp. include several genera resistant to heat. *Deinococcus radiodurans* is an extremophilic bacterium, and is the most radioresistant organism known. It can survive heat, cold, dehydration, vacuum, and acid, and because of its resistance to more than one extreme condition, *D. radiodurans* is known as a polyextremophile.

   Thermus aquaticus is important in the development of the polymerase chain reaction (PCR) where repeated cycles of heating DNA to near boiling make it advantageous to use a thermostable DNA polymerase enzyme. These bacteria have thick cell walls that give them Gram-positive stains, but they include a second membrane and so are closer in structure to those of Gram-negative bacteria.
6. *Spirochetes*
   Spirochetes are Gram-negative bacteria, which have long, helically coiled cells. Spirochetes are chemoheterotrophic in nature, with lengths between 5 and 250 μm and diameters around 0.1–0.6 μm. Spirochetes are distinguished from other bacterial phyla by the presence of flagella, sometimes called *axial filaments*, running lengthwise between the cell membrane and an outer membrane. These cause a twisting motion which

allows the spirochete to move about. The spirochete shape may also be described as consisting of an axial filament around which the cell is wound giving spirochetes their characteristic corkscrew shapes. Most spirochetes are free-living and anaerobic, but they also include the following disease-causing members:

- *Leptospira* species, which causes leptospirosis (also known as Weil's disease)
- *Borrelia burgdorferi*, which causes Lyme disease
- *Borrelia recurrentis*, which causes relapsing fever
- *Treponema pallidum*, which causes syphilis

7. *Green sulfur bacteria*

Green Sulfur Bacteria are found in anaerobic environments such as muds, anaerobic, and sulfide-containing fresh or marine waters, and wetlands. These anoxygenic phototrophic bacteria live in environments where light and reduced sulfur compounds are present. They are found most often under the *Purple Sulfur* bacterial layer. Green sulfur bacteria are capable of using sulfide or elemental S as the electron donor. The elemental S arises from H_2S oxidation and is deposited extracellularly, before the oxidation of sulfate. There are four genera of green sulfur bacteria, *Chlorobium*, *Prosthecohloris* (with stalks or prostheca), *Pelodictyon* (with vacuoles), and *Clathrochloris* (motile).

The *Green Sulfur Bacteria* strains are green because of the presence of bacteriochlorophylls (bchls) "c" and "d" and small traces of bchl "a" located in chlorobium vesicles attached to the cytoplasmic membrane. Some are brown and they contain bacteriochlorophyll "e." These brown strains are found in the deeper layers of wetlands and water. Both of the two groups can be found also living in extreme conditions of salinity and high temperatures. The morphology of both color types is most often either straight or curved rods.

They are non-motile phototrophic short to long rods which utilize H_2S as electron donor oxidizing it to SO_2 and to SO_4^{2+}. The sulfur so produced lies outside the cells. Light energy absorbed by Bacteriochlorophylls c, d, or e is channeled to Bacteriochlorophyll a, which actually carries out photosynthetic energy conversion, and ATP synthesis takes place. A well-known member is *Chlorobium tepidum*.

In marine environments, they are found in the water column where hydrogen sulfide diffuses up from anaerobic sediments and where oxygen diffuses down from surface waters where oxygenic photosynthesis is taking place. In the Black Sea, the largest anoxic water body in the world, they are found at a depth of 100 m (Manske et al. 2008). They also live in special tissues in invertebrates such as *Riftia pachyptila* (vestimentiferan tube worms) and *Calyptogena magnifica* ("giant" white clams) that live around deep sea hydrothermal vents. There they provide energy, by oxidizing reduced sulfur compounds, and organic matter, by converting carbon dioxide to organic compounds, which the invertebrates use. They are sometimes abundant in coastal waters, and several members of the group have gas vacuoles in their cells to help them float.

8. *Flavobacteria*

Flavobacteria are Gram negative rods that are motile by gliding and found in aquatic environments, both freshwater and marine, and in the soil. Colonies are usually yellow to orange in color, hence their name. Flavobacteria are a group of commensal bacteria and opportunistic pathogens. *Flavobacterium psychrophilum* causes the septicemic diseases of rainbow trout fry syndrome and bacterial cold water disease. They decompose several polysaccharides including agar but not cellulose. The type species is *F. aquatile*.

9. *Defferibacter*

These are thermophilic, anaerobic, chemolithoautotrophic Gram negative straight to bent rods. They can use a wide range of electron acceptors including Fe^{3+} and Mn^{2+}. They are found in a wide range of aquatic environments including deep-sea hydrothermal vents. A well-known member is *Deferribacter desulfuricans*

10. *Cytophaga*

Cytophaga are unicellular, Gram-negative gliding bacteria. They are rod-shaped, but specific strains differ in diameter and length with some being pleomorphic (many shaped). The type species is *C. johnsonae*, which has a moderately long thin shape. Many strains are red, yellow, or orange because of unique pigments synthesized by the group. *Cytophaga* strains tend to be versatile in making these and one strain may synthesize 25 different structural varieties of pigment. The main habitats of *Cytophaga* are soils at or close to neu-

tral pH, decaying plant material, and dung of animals. In freshwater environments, they are found on riverbanks and lake shores, in estuaries, bottom sediments, and algal mats. They are also common in sewage treatment plants, especially at the latter stages where only recalcitrant molecules remain. *Cytophaga* tend to degrade polymers such as cellulose and have been shown to be the major cellulose degraders in some lakes. A few species have been isolated from the oral cavity of humans where they appear to be part of the normal flora, but can occasionally cause septicemias. Some *Cytophaga* strains are pathogens of fish.

11. *Planctomyces/Pirella*
Planctomyces, *Pirella*, *Gemmata,* and *Isosphaera* form a phylogenetically related group of microorganisms that have many unusual properties. They are the only bacteria, other than the confusing case of the *Chlamydia*, whose cells lack peptidoglycan. Cells of this group divide by budding. Some members of the group produce long appendages, called stalks, and new cells are motile, developing stalks as they mature. Some members of this group have structures resembling nuclear membranes (Bauld and Staley 1976): others have fimbrin.

    Cells of this group can be pigmented (light rose, bright red, or yellow to ochre) or non-pigmented. An example of the species is *Planctomyces limnophilus*, which is ovoid, has a diameter of 1.5 μm, and forms red pigmented colonies. It grows slowly at temperatures between 17°C and 39°C and takes at least a week to form colonies. Stalks of the organism are very thin and cannot be seen by light microscopy. These stalks appear to be made of thin fibers twisted into a bundle that emanates from one pole of the ovoid cell. Cells multiply by budding and new cells are motile and stalkless, but eventually grow stalks as part of a maturation process similar to that seen for *Caulobacter.*

    These microbes are common inhabitants of freshwater lakes, marine habitats, and salt ponds, but most have been difficult to isolate in pure culture. For example, three of the four species in *Planctomyces* have only been observed in lake water and never isolated.

12. *Verrucomicrobia*
Verrucomicrobia, with the best example as *Verrucomicrobia spinosum*, has been isolated from freshwater, soil environments, and human feces. It produces cytoplasmic appendages called prostheca. Prostheca are like warts and the name of the group comes from the Greek word for warts. Both mother and daughter contain prostheca at the time of the cell division.

13. *Chlamydia*
Chlamydia are obligate intracellular pathogens with poor metabolic capabilities. They cannot synthesize biomolecules such as amino acids which they obtain from their hosts. Many Chlamydiae coexist in an asymptomatic state within specific hosts, and it is widely believed that these hosts provide a natural reservoir for these species.

    Chlamydiae exist in two states: a metabolically inert *elementary body* (EB) and a metabolically active *reticulate body* (RB) found only inside cells. EB is similar to the virions of viruses and enters the body by phagocytosis. Once ingested and inside the cell, EB divides and becomes RB. After it has killed the cell, it becomes EB again and is ready to be transmitted. Chlamydiae are spread by aerosol or by contact and require no alternate vector.

    Diseases caused by Chlymidia include sexually transmitted infections (STIs) (*Chlamydia trachomatosis*), pneumonia (*Chlamydia pneumoniae*), and bird pneumonia (*Chlamydia psittaci*).

14. *Cyanobacteria*
Cyanobacteria (Greek: κυανός (*kyanós*) = blue + bacterium) obtain their energy through photosynthesis. They are often referred to as blue-green algae, because they were once thought to be algae. They are a significant component of the marine nitrogen cycle system and an important primary producer in many areas of the ocean. Their ability to perform oxygenic (plant-like) photosynthesis is thought to have converted the reducing atmosphere of the early earth into an oxidizing one, which dramatically changed the life forms on Earth and provoked an explosion of biodiversity.

    Cyanobacteria are found in almost every conceivable habitat, from oceans to freshwater to bare rock to soil. Most are found in freshwater, while others are marine, occur in damp soil, or even temporarily moistened rocks in deserts. A few are endosymbionts in lichens, plants, various protists, or sponges and provide energy for the host. Some live in the fur of sloths, providing a form of camouflage.

    Cyanobacteria include unicellular and colonial species. Colonies may form filaments, sheets, or

even hollow balls. Some filamentous colonies show the ability to differentiate into several different cell types: vegetative cells, the normal, photosynthetic cells that are formed under favorable growing conditions; akinetes, the climate-resistant spores that may form when environmental conditions become harsh; and thick-walled heterocysts, which contain the enzyme nitrogenase, vital for nitrogen fixation. Heterocysts may also form under the appropriate environmental conditions (anoxic) wherever nitrogen is necessary. Heterocyst-forming species are specialized for nitrogen fixation and are able to fix nitrogen gas, which cannot be used by plants, into ammonia (NH_3), nitrites (NO_2^-), or nitrates (NO_3^-), which can be absorbed by plants and converted to protein and nucleic acids. The rice paddies of Asia, which produce about 75% of the world's rice, do so because of the high populations of nitrogen-fixing cyanobacteria in the rice paddy fields.

Photosynthesis in cyanobacteria generally uses water as an electron donor and produces oxygen as a by-product, though some may also use hydrogen sulfide as is the case among other photosynthetic bacteria. Carbon dioxide is reduced to form carbohydrates via the Calvin cycle. In most forms, the photosynthetic machinery is embedded into folds of the cell membrane, similar to thylakoids found in the chloroplasts of higher plants.

The cyanobacteria are traditionally classified by morphology into five sections, I–V: Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales, and Stigonematales. The latter two contain hetrocysts. The members of Chroococcales are unicellular and usually aggregated in colonies. In Pleurocapsales, the cells have the ability to form internal spores (baeocytes). In Oscillatorialles, the cells are singly arranged and do not form specialized cells, (akinets and heterocysts). In Nostocalles and Stigonematalles, the cells have the ability to develop heterocysts under certain conditions.

15. *Gram positive bacteria (including Mycoplasmas and Actinobacteria)*

Like the Proteobacteria, the Gram positive bacteria are very diverse; they contain many bacteria encountered in everyday life as agents of disease and inputs of production in industry or as important organisms in food microbiology. Some of them (the Mycoplasma) lack cell walls.

Gram-positive bacteria fall into two major phylogenetic divisions, "low-G + C" and "high-G + C.":

(a) Low G + C group: G + C below 50%;
(b) High G + C group: G + C higher than 50%

(a) Low G + C Group: G + C Below 50%

Non-sporulating Low G + C Group

Staphylococcus: The staphylococci have spherical cells often found in groups resembling clusters of grapes. Bacteria of this genus were originally grouped with other spherical microorganisms, especially of the genus *Micrococcus*, since these two genera often shared similar habitats. However, physiological studies and phylogenetic analysis have shown that these two genera are very different from one another. The differences between staphylococci and micrococci are discussed below.

Lactic Acid Bacteria: The lactic acid bacteria are Gram-positive rods and cocci that produce lactic acid as their primary end product. An important group characteristic is the absence of cytochromes, porphyrins and respiratory enzymes. They are therefore incapable of oxidative phosphorylation or any type of respiration and are totally dependent on fermentation. Lactic acid bacteria do, however, contain mechanisms to deal with the toxic byproducts of oxygen, which categorizes them as aerotolerant anaerobes. They include *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, *Enterococcus*, and *Lactobacillus.* Lactic acid bacteria are primarily differentiated based on the types of end products they form. Homofermentative lactic acid bacteria produce only lactic acid as an end product, while heterofermentative lactic acid bacteria produce lactate, ethanol, and CO_2 as well (Axelssson and Ahrne 2000; Narayanan et al. 2004).

Sporulating Low G + C Group

Bacillus: These are spore-forming aerobic rods. *Bacillus* is a genus of rod-shaped, beta-hemolytic Gram-positive bacteria. *Bacillus* species are catalase-positive obligate or facultative aerobes. Ubiquitous in nature, *Bacillus* includes both free-living in soil, water and air, as well as some pathogenic species. Under

stressful environmental conditions, the cells produce endospores resistant to heat, radiation, chemicals and other unfavorable conditions.

Clostridium: These are Gram-positive spore-forming obligately anaerobic rods. Individual cells are rod-shaped, and the name comes from the Greek for spindle. *Clostridium* includes common free-living bacteria as well as important pathogens, including *C. botulinum*, an organism producing a very potent toxin in food; *C. difficile*, which can overgrow other bacteria in the gut during antibiotic therapy; *C. tetani*, the causative organism of tetanus; *C. perfringens*, formerly called *C. welchii*, which causes a wide range of symptoms, from food poisoning to gas gangrene. Because *C. perfringens* produces much gas, it is also used as a replacement for yeasts in breadmaking. *C. sordellii* has been linked to the deaths of more than a dozen women. They are important in the anaerobic conditions of muds.

Heliobacteria: Heliobacteria are strictly anaerobic, spore-forming photoheterotrophic members of the Firmicutes. 16s rRNA studies put them among the *Firmicutes* (*Bacillus* and *Clostridium*) but they do not stain Gram-positively like the other members. They have no outer membrane and like certain other firmicutes (clostridia), they form heat resistant endospores. They are the only firmicutes known to conduct photosynthesis. Soluble periplasmic components appear absent in heliobacteria and photosynthesis takes place at the cell membrane, which does not form folds or compartments as it does in purple phototrophic bacteria. A particularity of heliobacterial photosynthesis is the occurrence of a unique Bacteriochlorophll (BChl) g. BChl *g* is chemically closer to Chl *a* than to BChl *a*. Correspondingly, heliobacteria appear to be more closely related to oxygenic photosynthesis than the green sulfur bacteria (based on 16S-rRNA phylogeny as well as on trees built from sequences of the photosynthetic reaction center). A small group, it is the only known phototrophic one among the Gram positives. Heliobacteria consist of three genera, *Heliobacterium* (3 spp.), *Heliobacillus* (1 sp.), and *Heliophilum* (1 sp.). They cannot tolerate sulfide, all known species can fix nitrogen. They are common in the waterlogged soils of paddy fields.

(b) High G + C Group: G + C Above 50%
These include Actinomycetes, Mycobacteria, Micrococcus, and Corynebacterium:
Actinomycetes
Actinomycetes are filamentous and spore-forming (non heat resistant spores), found in soil. They are very important as antibiotic producers. Typical example is *Streptomyces* sp. They include some of the most common soil life, playing an important role in decomposition of organic materials, such as cellulose and chitin and thereby playing a vital part in organic matter turnover and carbon cycle. Actinomycetes of the family Actinoplanaceae, especially *Actinoplanes*, are readily isolated from the flowing waters of rivers and streams, where they are important in the decomposition of wood and other cellulolisic materials.

Mycobacterium
This is a slow-growing acid-fast strain (Ziel–Nieelsen stain) implicated in diseases (*M. leprae*, leprosy; *M. tuberculosis*, tuberculosis). Many are however free-living and inhabit aquatic environments. These environmental or waterborne mycobacteria (WBM) inhabit a diverse range of natural environments and are a frequent cause of opportunistic infection in human beings and livestock. Several hospital and community outbreaks of mycobacterial infections, including infections as diverse as life-threatening pneumonia in patients with artificial ventilation, cystic fibrosis, and chronic granulomatous disease; outbreaks of skin infection following liposuction; furunculosis after domestic footbaths; mastitis after body piercing; and abscess formation in intravenous drug users.

Corynebacteria
Corynebacterium is a genus of Gram-positive, facultatively anaerobic, nonmotile, rod-shaped actinobacteria. Most do not cause disease, but are part of normal human skin flora. *Corynebacterium diphtheriae* is the cause of diphtheria

Table 4.7 Properties of Micrococci and Staphylococci

Species	Cells arrangement	G + C ratio	Oxygen requirement
Micrococcus	Clusters, tetrads (fours)	66–73	Strictly aerobic
Staphylococcus	Clusters, pairs	30–39	Microaerophilic

in humans. The genes encoding exotoxins that are the cause of diphtheria (caused by Corynebacterium) as well as cholera and some other bacterial diseases are mobile in aquatic and terrestrial environments and have been found in sediments and in river water using PCR.

Micrococcus
These are cocci in bunches and very similar to staphylococci. They are distinguished from each other according to the properties shown in Table 4.7.

16. *Nitrospira*
Nitrospira are nitrite-oxidizing bacteria that are important in marine habitats. In aquaria, for example, if the ammonia/nitrite/nitrate cycle is exhausted, the ecosystem suffers and fish can get sick or die. Therefore, nitrite-oxidizing bacteria as well as the other bacteria in this system are important for healthy marine ecosystems. In addition, *Nitrospira*-like bacteria are the main nitrite oxidizers in wastewater treatment plants.
17. *Proteobacteria (including purple bacteria)*
The *Proteobacteria* are a major group of bacteria. They include a wide variety of pathogens, such as *Escherichia*, *Salmonella*, *Vibrio*, and *Helicobacter*. Others are important agriculturally or industrially; still others are free-living, and include many of the bacteria responsible for nitrogen fixation. The group is defined primarily in terms of ribosomal RNA (rRNA) sequences, and is named after the Greek god Proteus, who could change his shape, because of the great diversity of forms found within the group. Proteus is also the name of a bacterial genus within the Proteobacteria.

All Proteobacteria are Gram-negative, with an outer membrane mainly composed of lipopolysaccharides. Many move with flagella, but some are nonmotile or move by bacterial gliding. The latter include the myxobacteria, a unique group of bacteria that can aggregate to form multicellular fruiting bodies.

There is also a wide variety in the types of metabolism. Most members are facultatively or obligately anaerobic and heterotrophic, but there are numerous exceptions. A variety of genera, which are not closely related to each other, convert energy from light through photosynthesis. These are called purple bacteria, referring to their mostly reddish pigmentation.

The Proteobacteria are divided into five sections, referred to by the Greek letters alpha through epsilon, again based on rRNA sequences.

Alpha (α) Proteobacteria
The Alphaproteobacteria comprise the most phototrophic genera, but also several genera metabolizing C1-compounds (compounds with a single carbon atom e.g., *Methylobacterium*, symbionts of plants (e.g., Rhizobia) and animals, and a group of intracellular pathogens, the Rickettsiaceae. Moreover, the precursors of the mitochondria of eukaryotic cells are thought to have originated in this bacterial group.

Beta (β) Proteobacteria
The Betaproteobacteria consist of several groups of aerobic or facultative bacteria which are often highly versatile in their degradation capacities, but also contain chemolithotrophic genera (e.g., the ammonia-oxidizing genus *Nitrosomonas*) and some phototrophs (genera *Rhodocyclus* and *Rubrivivax*). Beta Proteobacteria play an important role in nitrogen fixation in various types of plants, oxidizing ammonium to produce nitrite- an important chemical for plant function. Many of them are found in environmental samples, such as waste water or soil. Pathogenic species within this class are the *Neisseriaceae* (gonorrhea and meningoencephalitis) and species of the genus *Burkholderia*.

Gamma (γ) Proteobacteria
The Gammaproteobacteria comprise several medically and scientifically important groups of bacteria, such as the Enterobacteriaceae, Vibrionaceae, and Pseudomonadaceae. Many important pathogens belong to this class, e.g., *Salmonella* (enteritis and typhoid fever), *Yersinia* (plague), *Vibrio* (cholera), *Pseudomonas aeruginosa* (lung infections in hospitalized or cystic fibrosis patients), and *E coli*.

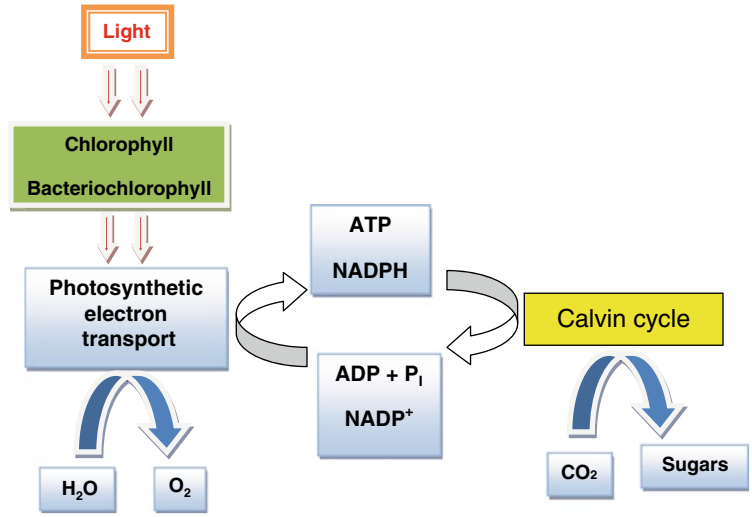


Fig. 4.11 Overview of photosynthesis (Modified from Moroney and Ynalvez 2009)

Delta (δ) Proteobacteria)

The Deltaproteobacteria comprise a group of predominantly aerobic genera, the fruiting-body-forming myxobacteria, and a branch of strictly anaerobic genera, which contains most of the known sulfate-reducing bactria, (*Desulfovibrio*, *Desulfobacter*, *Desulfococcus*, *Desulfonema*, etc.) and sulfur-reducing bacteria (e.g., *Desulfuromonas*) alongside several other anaerobic bacteria with different physiology (e.g., ferric iron-reducing *Geobacter* and *Pelobacter* and *Syntrophus* species, which live symbiotically together).

Epsilon (ε) Proteobacteria

The Epsilonproteobacteria consist of only a few genera, mainly the curved to spiral-shaped *Wolinella*, *Helicobacter*, and *Campylobacter*. Most of the known species inhabit the digestive tract of animals and humans and serve as symbionts (*Wolinella* in cows) or pathogens (*Helicobacter* in the stomach and *Campylobacter* in the duodenum in humans). There have also been numerous environmental sequences of epsilons recovered from hydrothermal vent and cold seep habitats.

4.1.4.2 Aspects of the Physiology and Ecology of Microorganisms in the Aquatic Environment

This section will discuss the physiology of some of the activities of aquatic microorganisms which contribute to their ecology in bodies of water as well as to their economic importance. Items to be discussed are photosynthesis, nitrogen economy, especially nitrogen fixation, sulfate reduction, and iron in the aquatic environment.

Photosynthesis

Photosynthesis is the conversion of CO_2 to carbohydrates using light energy. This process has been described as the most important biological reaction on earth, since it is the means by which the energy of the sun is harnessed by living things, through their consumption of the products of photosynthesis. Photosynthesis is carried out by plants, algae, and some bacteria, but not by Archae. It is an important factor affecting the ecology of microorganisms in aquatic environments (Achenbach et al. 2001). Photosynthesis is generally better known in plants than in bacteria; plant photosynthesis will therefore be discussed as a basis for understanding bacterial photosynthesis (see Fig. 4.11).

Photosynthesis is hinged on three items: (a) *Photosynthetic pigments*, (b) the *light* or light-dependent reactions of photosynthesis, and (c) the *dark* or light-independent reactions of photosynthesis.

The Pigments of Photosynthesis

A pigment is any substance that absorbs light. The color of the pigment comes from the wavelengths of light reflected by the pigment (in other words, those not absorbed). Chlorophyll, the green pigment common to all photosynthetic cells, absorbs all wavelengths of visible light except green, which it reflects, and thus is detected by human eyes as green. Black pigments absorb all of the wavelengths that strike

them. White pigments/lighter colors reflect all or almost all of the light energy striking them. Pigments have their own characteristic absorption spectra, the absorption pattern of a given pigment.

Chlorophyll (chl) found in plants, algae, and cyanobacteria, is very similar to bacteriochlorophyll (bchl) found in bacteria, other than cyanobacteria (see Fig. 4.12). There are several types of chlorophylls and of bacteriochlorophylls, (named a, b, c, d, e, and g) differing from each in slight differences in structure. Bchls "a" and "b" are found in the purple bacteria; while bchls "c," "d," and "e" are found in Green sulfur bacteria; bchl "g" is found in *Heliobacteria*. In higher plants, photosynthesis takes place only in chl "a"; all other chlorophylls along with carotenoids are accessories and gather light which is channeled to chl "a." Similarly, in bacteria, bchl "a" is the site of photosynthesis; all the other bacteriochlorophylls are accessories and gather light which is channeled to bchl "a."

Accessory pigments include carotenoids found in higher plants and cyanobacteria and phycobilins found in the algae. Pigments have their own characteristic absorption spectra. Figure 4.13 shows the wavelength of various chlorophylls and accessory pigments.

The Light Reactions

In higher plants, the light dependent reactions, take place on membranous structures known as thylakoids found in chloroplasts in complex processes, that are not yet fully understood. The process is much simplified as described below.

In plants, light is absorbed by complexes formed between protein and chlorophyll molecules known as photosystems, Photosystem I (PSI) and Photosystem II (PSII). PSII absorbs light energy (photons) at a wavelength of 680 nm and is called P680 while PSI it absorbs photons at 700 nm and is called P700.

When a pigment absorbs light energy, one of three things will occur: Energy may be dissipated as heat; it may be re-emitted immediately as a longer wavelength, a phenomenon known as fluorescence; or the energy may trigger a chemical reaction, as in photosynthesis. In plant photosynthesis, the action begins at the PSII chlorophyll–protein complex which becomes excited and loses an electron; this electron is passed through a series of enzymes until it is transferred to water, causing it to lose electrons:

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2$$

The electron released from the splitting of water is transferred to PSI, which can itself capture light energy; this energy is transferred by enzymes used to reduce $NADP^+$ to NADPH and ATP the other energy currency of cells, thus ADP + Pi ⇒ ATP.

While the photosynthetic process in cyanobacteria is similar to that of plants, green bacteria and purple sulfur bacteria have photosynthetic processes different from the process in plants.

Cyanobacteria do not have chloroplasts, but have structures on their cell membranes which are similar to thylakoids. They have photosystems similar to PS II and PS I found in the chloroplasts of higher plants. They can produce NADPH and ATP in the way as higher plants and they are the only bacteria which produce O_2 during photosynthesis. However, instead of carotenoids or chlorophyll "b" which act as accessory pigments in higher plants, they have phycobilins.

Purple Bacteria: Purple bacteria and green sulfur bacteria have only one type of photosystem. The single photosystem in purple bacteria is structurally related to PS II in cyanobacteria and plant chloroplasts; it, however, has a P870 molecule, i.e., it absorbs light at 870 nm and can make ATP in the transfer of electrons.

In order to make NADPH, purple bacteria use an external electron donor (hydrogen, hydrogen sulfide, sulfur, sulfite, or organic molecules such as succinate and lactate) to feed electrons into a reverse electron transport chain.

Green Sulfur Bacteria: These bacteria contain a photosystem that is analogous to PS I (P840) in chloroplasts. It makes ATP through the transfer of electrons. Electrons are removed from an excited chlorophyll molecule and used to reduce NAD^+ to NADH. The electrons removed from P840 must be replaced. This is accomplished by removing electrons from H_2S, which is oxidized to sulfur which appear as globules in the cells (hence the name "green sulfur bacteria").

$$H_2S \Rightarrow 2H + S^0.$$

The Dark or Light-Independent Reactions of Photosynthesis

The energy rich ATP and NADPH molecules formed in the light dependent phase of photosynthesis are used

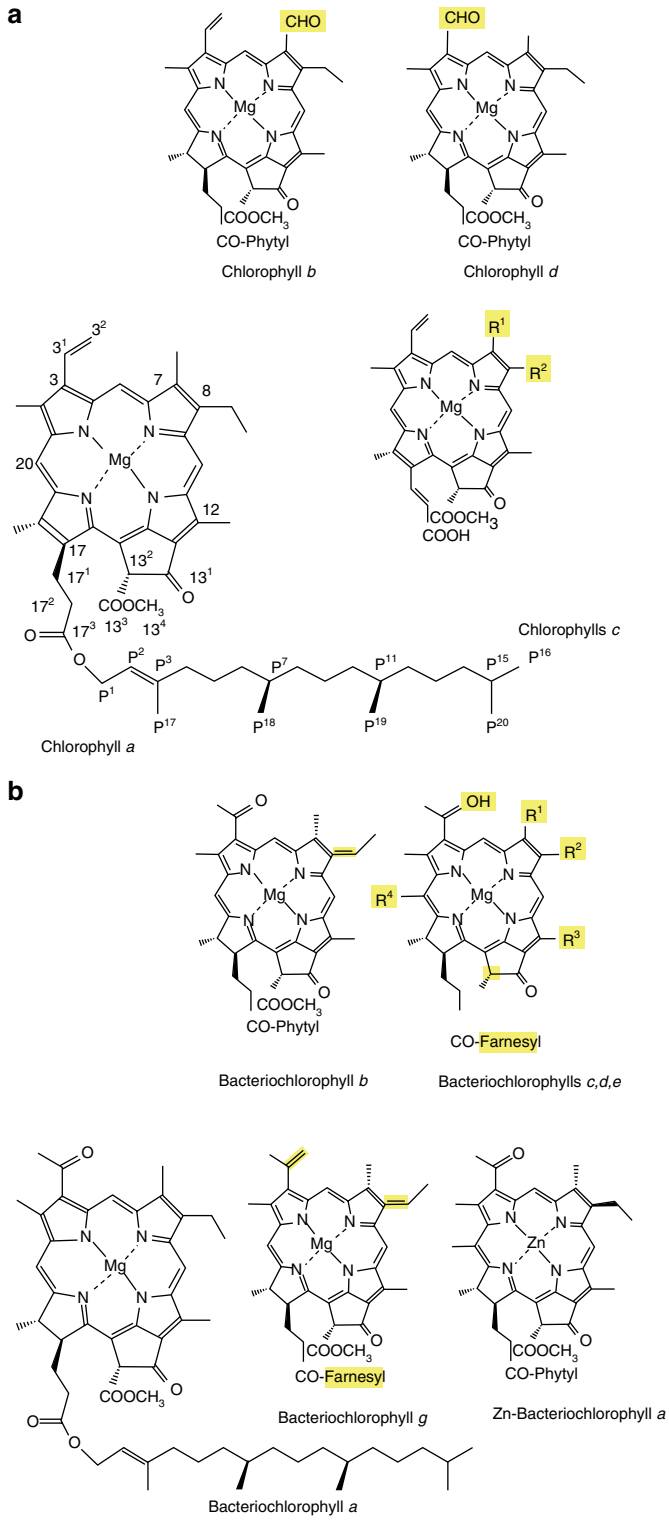


Fig. 4.12 Structure of chlorophylls and bacteriochlorophylls (From *Encyclopedia of Life Sciences*; Fujita 2005. With permission) Structure of various Chls (a) and BChls (b). (a) Chls a, b, c and d. Differences in side-chains of Chls b, c and d from Chl a are highlighted with yellow boxes. (b) BChls a, b, c, d, e, g and Zn-BChl a. Differences in sidechains, ring oxidation state, and the central metal ion of BChls b, c, d, e, g and Zn-BChl a are highlighted with yellow boxes.

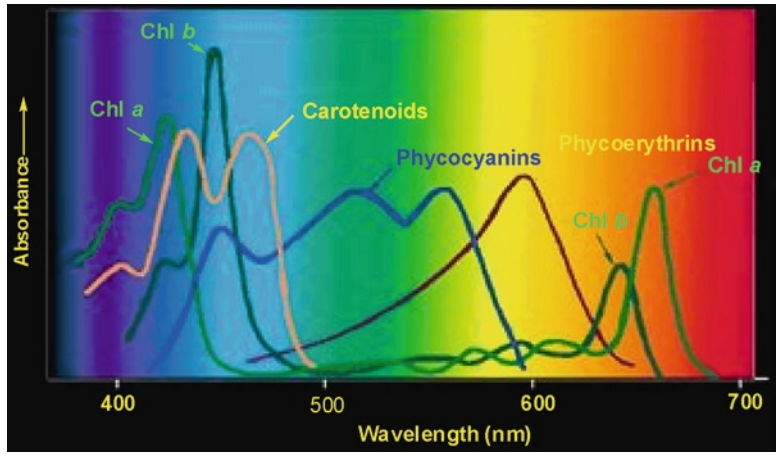


Fig. 4.13 Wavelengths of chorophylls and photosythetic accessory pigments (Modified from Photosynthesis: Light energy transduced to chemical energy; http://phototroph.blogspot.com/2006/11/pigments-and-absorption-spectra.html)

for the production of energy rich carbohydrates (sugars) in the Calvin cycle (see Fig. 4.11).

The fixation or reduction of carbon dioxide is a light-independent process in which carbon dioxide combines with a five-carbon sugar, ribulose 1,5-bis-phosphate (RuBP), to yield two molecules of a three-carbon compound, glycerate 3-phosphate (GP), also known as 3-phosphoglycerate (PGA). GP, in the presence of ATP and NADPH from the light-dependent stages, is reduced to glyceraldehyde 3-phosphate (G3P) and enters the citric acid cycle.

The processes of photosynthesis can be represented by the general formula:

$$CO_2 + 2H_2A \Rightarrow CH_2O + H_2O + 2A,$$

where H_2A is the source of the reducing power for the conversion of CO_2 to carbohydrates.

In higher plants, algae and cyanobacteria, where water is the source of the reducing power photosynthesis, can be represented thus:

$$CO_2 + 2H_2O \Rightarrow CH_2O + H_2O + O_2.$$

In bacteria, other than cyanobacteria, where water is not the source of reducing power and hence oxygen is not involved (anoxygenic), for example, the green sulfur bacteria, where hydrogen sulfide is utilized, the photosynthetic equation is given thus:

$$CO_2 + 2H_2S = CH_2O + H_2O + 2S.$$

Summary: Differences Between Photosynthesis in Plants and in the Bacteria

Like green plants, some bacteria are photosynthetic, using the energy of sunlight to reduce carbon dioxide to carbohydrate. There are a number of differences between the two groups which are summarized below:

1. *Chlorophyll and bacteriochlorophyll*
   Chlorophyll, the photosynthetic pigment in plants, is replaced in bacteria by bacteriochlorophyll (except in the Cyanobacteria). Both types of pigments are similar and differ only in some side chains (see Fig. 4.12).
2. *Sites for photosynthesis in green plants and bacteria*
   In higher plants, photosynthesis takes places in membraneous structures known as thylakoids which are located in organelles known as chloroplasts. In bacteria, the site for photosynthesis varies from one group of bacteria to the other. In the cyanobacteria, although chloroplasts are absent, photosynthesis occurs in thylakoid-like structures; in helicobacteria, it takes place on the cell membranes; in the purple bacteria, it takes place in invaginations of the cell membrane; in the green bacteria, it takes place on the cell membrane as well as in special membrane folding known as chlorosomes.
3. *Oxygenic and anoxygenic photosynthesis*
   In higher plants, algae and cyanobacteria, the light energy excites the molecules of chlorophyll leading to release of energy which splits the water molecule and to the release of oxygen as a by-product, and finally the provision of H for fixing the CO_2. In most

bacteria (apart from cyanobacteria), oxygen is not released because water does not provide the H which converts the CO_2 to carbohydrates. Rather, light energy excites bacteriochlorophyll leading to energy which splits H from H_2S. In the dark, many photosynthetic bacteria can produce energy by the transfer of electron, or anaerobically.

Aspects of the Physiology of Photosynthetic Bacteria

The photosynthetic bacteria can be divided into two groups: The anaerobic photosynthetic groups and the aerobic photosynthetic bacteria.

1. *The anaerobic photosynthetic bacteria (AnPB)*
   The bacterial order *Rhodospirillales* contains three photosynthetic families:
   (a) *Rhodospirillaceae*: Purple non-sulfur bacteria, e.g., *Rhodospirillum*. These cells contain bacteriochlorophyll "a" or "b" located on specialized membranes continuous with the cytoplasmic membrane. They are not able to use elemental sulfur as electron donor and typically use an organic electron donor, such as succinate or malate, but can also use hydrogen gas.
   (b) *Chromatiaceae*: These include purple sulfur bacteria, e.g., *Chromatium*. They are able to use sulfur and sulfide as the sole photosynthetic electron donor and sulfur can be oxidized to sulfate. They can use inorganic sulfur compounds, such as hydrogen sulfide as an electron donor. Purple sulfur bacteria must fix CO_2 to survive, whereas non-sulfur purple bacteria can grow aerobically in the dark by respiration on an organic carbon source. They store elemental sulfur inside their cells, and these appear globules within their cells, hence their name, purple *sulfur* bacteria.
   (c) *Chlorobiaceae*: These are green sulfur bacteria; their cells contain bacteriochlorophyll "c" or "d" located in chlorobium vesicles attached to the cytoplasmic membrane.
   (d) *Heliobacteria*: The heliobacteria are anaerobic and phototrophic, converting light energy into chemical energy by photosynthesis using a PSI type reaction center (RC) (P798). The primary pigment involved is bacteriochlorophyll *g*, which is unique to the group and has a unique absorption spectrum. On account of this, the heliobacteria occupy their own special environmental niche. Phototrophy takes place on the cell membrane, which does not form folds or compartments as it does in purple phototrophic bacteria. Using 16 S RNA analysis, they are placed among the Firmicutes, Gram positive bacteria; although, they do not stain Gram positive, but they form heat resistant endospores. Heliobacteria are the only firmicutes known to conduct photosynthesis. They are *photoheterotrophic*, i.e., they require organic carbon sources. They do not fix carbon dioxide, they lack rubisco, and do not have Calvin cycle.

   They are found in soils, especially water logged soils such as in paddy fields. They are also strong nitrogen fixers.
2. *The aerobic photosynthetic bacteria (APB)*
   The cyanobacteria are photosynthetic and aerobic, but recently another photosynthetic aerobic group was discovered. It was previously generally believed that anoxygenic photosynthesis was an anaerobic growth mode of either obligately anaerobic, or facultatively anaerobic bacteria capable of switching between respiration under aerobic conditions and phototrophy under anaerobic conditions. Recently (1979), the first reported member of the aerobic phototrophic bacteria, *Erythrobacter longus*, discovered in the Bay of Japan, changed our previous knowledge of the phototrophic bacteria. APBs have since been found in a wide variety of both marine and freshwater habitats, including acid mine drainage sites, soils, saline lakes, and soda lakes. (Rathgeber et al. 2004). Other genera of APBs found in freshwater and marine environments include the following: *Erythrobacter,*, *Roseobacter,*, *Porphyrobacter,*, *Acidiphilium Erythromonas*, *Erythromicrobium*, *Roseococcus,* and *Sandaracinobacter.*

   The distinguishing features of APBs are:
   (a) They produce their photosynthetic apparatus only in the presence of oxygen and the absence of light.
   (b) The presence of bacteriochlorophyll a (BChl a) incorporated into light harvesting (LH) and reaction center (RC) complexes capable of transforming light into electrochemical energy under aerobic conditions.
   (c) A relatively low amount of photosynthetic units per cell.
   (d) Extreme inhibition of BChl synthesis by light.
   (e) An abundance of carotenoid pigments.
   (f) Apparent lack of intracytoplasmic photosynthetic membranes.
   (g) The inability to grow phototrophically under anaerobic conditions.

The APBs produce a photosynthetic apparatus similar to that of purple phototrophic bacteria. However, this apparatus, in contrast to that of the anaerobic photosynthetic bacteria is produced only under aerobic conditions. In facultatively anaerobic organisms, the photosynthetic apparatus is synthesized under conditions of oxygen shortage and absence of light.

They are a phylogenetically diverse group interspersed predominantly throughout the α-Proteobacteria, closely related to anoxygenic phototrophic purple non-sulfur bacteria as well as chemotrophic species. Recently, however, more and more APBs have been placed in the β-Proteobacteria.

Nitrogen Economy in Aquatic Systems

Nitrogen Fixation

Nitrogen is important in microorganisms for the manufacture of proteins and nucleic acids, both of which are essential for the continued existence of all living things. Although the element is abundant in the atmosphere, constituting about 80%, the ability of making atmospheric nitrogen available to living things is present only in a few organisms. Biological nitrogen fixation can be represented by the following equation, in which 2 moles of ammonia are produced from 1 mole of nitrogen gas, at the expense of 16 moles of ATP and a supply of electrons and protons (hydrogen ions):

$$N_2 + 8H^+ + 8e^- + 16\ ATP = 2NH_3 + H_2 + 16ADP + 16\ Pi.$$

This reaction is performed exclusively by prokaryotes using a nitrogenase enzyme complex. This enzyme consists of two proteins – an iron protein and a molybdenum-iron protein – as shown Fig. 4.14.

The reactions occur while N_2 is bound to the nitrogenase enzyme complex. The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum–iron protein, which donates electrons to N_2, producing HN=NH. In two further cycles of this process (each requiring electrons donated by ferredoxin), HN=NH is reduced to $H_2N–NH_2$, and this in turn is reduced to $2NH_3$. Depending on the type of microorganism, the reduced ferredoxin, which supplies electrons for this process, is generated by photosynthesis, respiration, or fermentation (Anonymous 2010e).

Nitrogen fixation may be done by bacteria living symbiotically with higher plants such as *Rhizobium* spp. and legumes or by free-living organisms. In the aquatic environment, the nitrogen fixers are free-living microorganisms including aerobic and anaerobic ones. (Naqvi 2006). Among aerobes, nitrogen fixers include all members of *Azotobacter* and *Beijerinckia,*, some *Klebsiella* and some cyanobacteria. Under anaerobic conditions, such as, occur in sediments or in the deeper regions of water columns, the following organisms fix nitrogen: Some *Clostridium* spp., *Desulfovibrio*, purple sulfur bacteria, purple non-sulfur bacteria, and green sulfur bacteria.

The nitrogenase enzyme complex is highly sensitive to oxygen and it is inactivated when exposed to oxygen, because oxygen reacts with the iron component of the proteins. Aerobic organisms including cyanobacteria, which produce oxygen during photosynthesis, combat the problem of nitrogenase inactivation by different methods. Cyanobacteria for example have special cells, heterocysts, where nitrogen fixation occurs and in which nitrogenase is protected because they contain only photosystem I whereas the other cells have both photosystem I and photosystem II (which generates oxygen when light energy is used to split water to supply H_2 in photosynthesis.). For the same reason, also, *Azotobacter* and *Rhizobium* produce large amounts of extracellular polysaccharide, which helps limit the diffusion of oxygen to the cells. Furthermore, *Rhizobium* root nodules contain oxygen-scavenging molecules such as leghemoglobin, which regulate the supply of oxygen to the nodule tissues in the same way as hemoglobin regulates the supply of oxygen to mammalian tissues.

Other microbial activities which participate in regulating the nitrogen economy of aquatic systems are *nitrification* and *denitrification*.

Nitrification

Nitrification is the conversion of ammonium to nitrate by the nitrifying bacteria. These bacteria are chemoautothrophs which obtain energy by oxidizing ammonium, while using CO_2 as their source of carbon to synthesize organic compounds. The nitrifying bacteria are found in most soils and waters of moderate pH, but are not active in highly acidic soils. They almost always are found as mixed-species communities or *consortia.* Some of them – e.g., *Nitrosomonas* convert ammonium to nitrite (NO_2^-) while others – e.g., *Nitrobacter* convert nitrite to nitrate (NO_3^-). The nitrifying bacteria are so numerous in waters rich in ammonium such as sewage

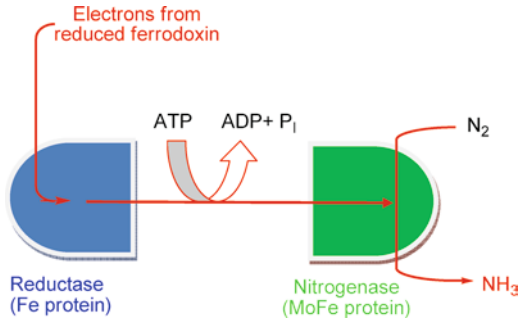


Fig. 4.14 The enzymes of nitrogen fixation (Reproduced from Berg et al. 2002. With permission)

Note: Ferrodoxins are a group of red-brown proteins containing iron and sulfur, which act as electron carriers during photosynthesis, nitrogen fixation, or oxidation-reduction reactions in green plants, algae, and anaerobic bacteria.

Nitrogenase is a two-protein complex. One component, **nitrogenase reductase** is an iron-containing protein that accepts electrons from ferredoxin, a strong reductant, and then delivers them to the other component, **nitrogenase**, which contains **Iron (Fe) and molybdenum (Mo)**.

The overall reaction in nitrogen fixation via nitrogenase is:

$$8H^+ + N_2 + 8e^- + 16ATP + 16H_2O \rightarrow$$
$$2NH_3 + H_2 + 16ADP + 16P_i + 16H_+$$

effluents that they readily convert the ammonium compounds therein into nitrates. The nitrates can accumulate in groundwater, and may ultimately enter drinking water. Regulations in many countries control the amount of nitrate in drinking water, because in the anaerobic conditions of the animal alimentary canal, nitrates can be reduced to highly reactive nitrites by microorganisms. Nitrites are absorbed from the gut and bind to hemoglobin, reducing its oxygen-carrying capacity. In young babies, this can lead to a respiratory illness known as *blue baby syndrome*. Nitrites can also react with amino compounds, forming nitrosamines which are highly carcinogenic.

Denitrification

Denitrification is the conversion of nitrate to gaseous compounds (nitric oxide, nitrous oxide, and N_2) by microorganisms. Denitrification goes through some combination of the following intermediate forms:

$$\underset{\text{nitrate}}{NO_3^-} \rightarrow \underset{\text{nitrite}}{NO_2^-} \rightarrow \underset{\text{nitric oxide}}{NO} \rightarrow \underset{\text{nitrous oxide}}{N_2O} \rightarrow \underset{\text{gas}}{N_2} \uparrow$$

The denitrification process can be expressed in terms of electron transfer thus:

$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O.$$

Denitrification is brought by a large number of different bacteria which are mainly heterotrophic. They complete the nitrogen cycle by returning N_2 to the atmosphere. Denitrification occurs under special conditions in both soil and aquatic conditions, including marine environments. Denitrification occurs when oxygen supply is low such as in ground water, wetlands in seafloors, and other poorly aerated parts of aquatic systems. The conditions which encourage denitrification are those in which there is a supply of oxidizable organic matter, and absence of oxygen and the availability of reducible nitrogen sources. Under such conditions, the terminal electron acceptor for the denitrifying bacteria is not oxygen but the nitrogen compounds given in the formula above. The organisms prefer nitrates and the other compounds in the equation, in the order they occur in the equation above and ending with nitrous oxide. When the terminal electron acceptor is an inorganic compound such as those in the formula above, the condition is also termed respiration as is also the case with oxygen.

A mixture of gaseous nitrogen products is often produced because of the stepwise use of nitrate, nitrite, nitric oxide, and nitrous oxide as electron acceptors in anaerobic respiration. The commonest denitrifying bacteria include several species of *Pseudomonas*, *Alkaligenes*, *Bacillus,*, and *Paracoccus denitrificans.* Autotrophic denitrifiers (e.g., *Thiobacillus denitrificans*) have also been identified. In general, however, several species of bacteria are involved in the complete reduction of nitrate to molecular nitrogen, and more than one enzymatic pathway have also been identified.

Anammox

In some organisms, direct reduction from nitrate to ammonium (also known as dissimilatory nitrate reduction to ammonium or DNRA) may also occur; although, this is less common than denitrification. Anammox, an abbreviation for ANaerobic AMMonium OXidation, is a globally important microbial process of the nitrogen cycle. It takes place in many natural environments.

The bacteria mediating this process were identified only 20 years ago. They belong to the bacterial phylum *Planctomycetes*, of which *Planctomyces* and *Pirellula* are the best known genera. Four genera of anammox bacteria have been identified: *Brocadia*, *Kuenenia*, *Anammoxoglobus*, *Jettenia* (all freshwater species), and *Scalindua* (marine species).

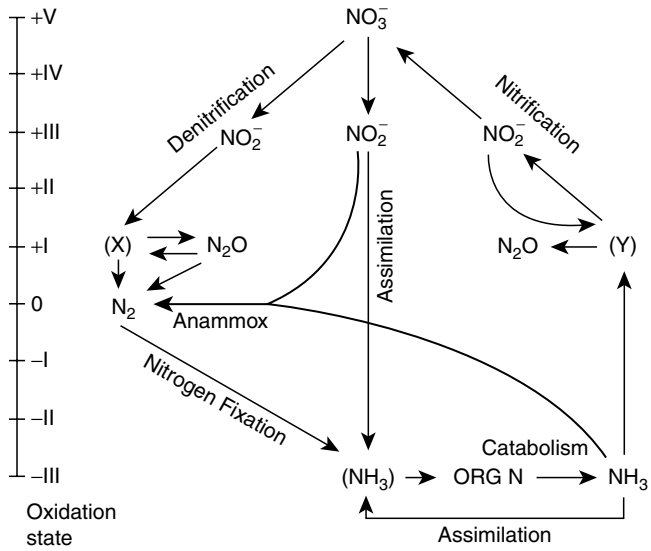


Fig. 4.15 Nitrogen cycle in the marine environment (From Codispoti et al. 2001. With permission) "X" and "Y" are intracellular intermediates that do not accumulate in water column

The anammox bacteria are characterized by several striking properties:

(a) They all possess one anammoxosome, a membrane bound compartment inside the cytoplasm which is the site of anammox catabolism.
(b) Further, the membranes of these bacteria mainly consist of ladderane lipids which are rare in living organisms.
(c) Hydrazine (normally used as a high-energy rocket fuel, and poisonous to most living organisms) is a by-product of these organisms.
(d) Finally, the organisms grow very slowly, the generation or doubling time being nearly 2 weeks.

The anammox process was originally found to occur only from 20°C to 43°C, but more recently, anammox has been observed at temperatures from 36°C to 52°C in hot springs and 60°C to 85°C at hydrothermal vents located in the ocean floor.

Reduction under anaerobic conditions can also occur through anaerobic ammonia oxidation (Anammox) thus:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O.$$

Because denitrifying bacteria are principally heterotrophic, in some wastewater treatment plants, small amounts of methanol are added to the wastewater to provide a carbon source for the bacteria.

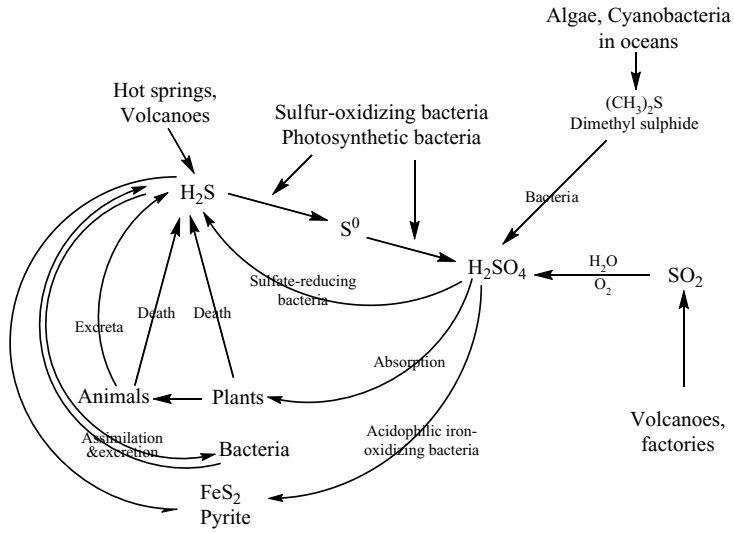
Nitrogen fixation, nitrification, and denitrification are interlinked in the nitrogen cycle. The nitrogen cycle in the marine environment is given in Fig. 4.15.

The Sulfur Cycle in the Aquatic System and Bacteria

In the environment, through changes brought about mostly by bacteria, sulfur changes from one form to the other: From hydrogen sulfide (H_2S) to sulfate via elemental sulfur (S^0) and sulfate is changed again to hydrogen sulfide. Hydrogen sulfide is also evolved from hot springs and volcanoes, and occurs when dead animals, the excreta of animals, and dead plants are decomposed by bacteria. The compound is oxidized to sulfuric acid by the sulfur-oxidizing bacteria and photosynthetic sulfur bacteria via elemental sulfur. The change of hydrogen sulfide to elemental sulfur occurs also abiotically in the presence of molecular oxygen.

Dimethyl sulfide $(CH_3)_2S$ is produced by marine algae and marine cyanobacteria and contributes to the typical smell of thee sea. Dimethyl sulfide is degraded by bacteria such as *Thiobacillus* and *Hyphomicrobium*, leading to the formation of acid. The various transformations are summarized in Fig. 4.16. A major group of bacteria important in the global economy of sulfur, especially in aquatic environments are the sulfate reducing bacteria. They will be discussed briefly below.

The sulfate reducing bacteria (SRB) are ubiquitous anaerobes found in diverse environments. They include several groups of bacteria that use sulfate as an oxidizing agent, reducing it to sulfide (Fig 4.17) (Luptakova 2007). They can also utilize other sulfur compounds, including sulfite, thiosulfate, and elemental sulfur in a type of metabolism known as dissimilatory, because



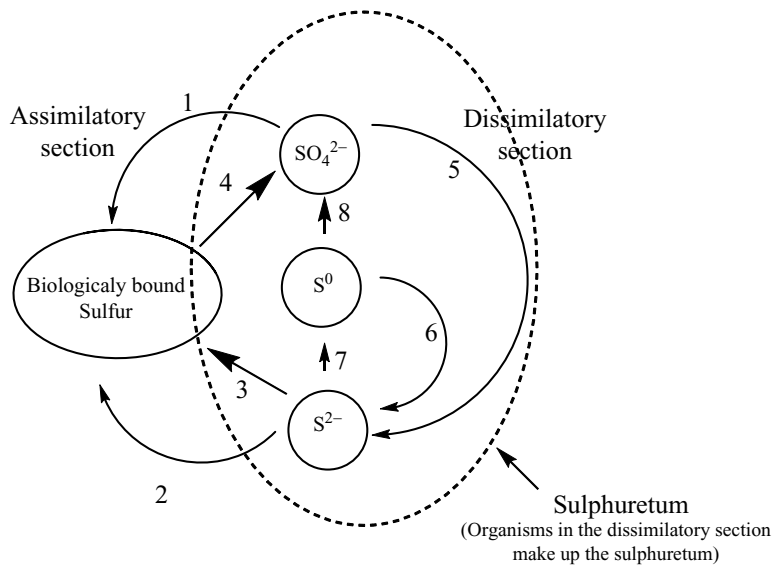


Fig. 4.16 General circulation of sulfur on Earth (Modified from Yamanaka 2008)

Fig. 4.17 The biological sulfur cycle (After Luptakova 2007). Key: 1 – Assimilatory sulfate reduction by plants, fungi and bacteria; 2 – Death and decomposition by fungi and bacteria; 3 – Sulfide assimilation by bacteria and some plants; 4 – Excretion of sulfate by animals; 5 – Dissimilatory sulfate-reducing bacteria; 6 – Dissimilatory sulfur-reducing bacteria; 7 – Phototrophic and chemotrophic sulfide-oxidizing bacteria; 8 – Phototrophic and chemotrophic sulfur-oxidizing bacteria

sulfur is not converted into organic compounds. The rotten egg odor of hydrogen sulfide in the environment usually indicates the presence of sulfate-reducing bacteria in nature. Sulfate-reducing bacteria are responsible for the rotten egg odors of salt marshes, mud flats, and intestinal gas. They slowly degrade materials that are rich in cellulose in anaerobic environments. Apart from soil, sulfate reducing bacteria are found in various habitats such as seas and oceans, mud and sediments of freshwaters (rivers, lakes), waters rich in decaying organic material, thermal or nonthermal sulfur springs, mining waters from sulfide deposits, waters from deposits of mineral oil and natural gas, industrial waste waters from metallurgical industry, as well as in the gastrointestinal tract of man and animals (Barton and Hamilton 2007).

Based on the energy source of sulfate reducing bacteria, there are two types of anaerobic respiration of sulfates: autotrophic and heterotrophic.

1. *Autotrophic reduction of sulfates*: In this case, the energy source is gaseous hydrogen; the reaction proceeds in several stages and the whole process can be expressed by:

$$4H_2 + SO_4^{2-} \xrightarrow{SRB} S^{2-} + 4H_2O \qquad (4.1)$$

2. *Heterotrophic reduction of sulfates*: The energy sources in heterotrophic reduction are simple organic substances (lactate, fumarate, pyruvate, some alcohols, etc.). The organic substrate may be incompletely or completely oxidized as shown in the two reactions given below:

(a) Incomplete heterotrophic oxidation of the organic substrate (acetate in this example):

$$2\,CH_3CHOHCOO^- + SO_4^{2-} \xrightarrow{SRB} 2\,CH_3COO^- + 2\,HCO_3^- + H_2S \qquad (4.2)$$

(b) Complete heterotrophic oxidation of organic substrate in which the final products are CO_2 and H_2O (Eq. 4.3):

$$4\,CH_3COCOONa + 5\,MgSO_4 \xrightarrow{SRB} 5\,MgCO_3 + 2\,Na_2CO_3 + 5H_2S + 5\,CO_2 + H_2O \qquad (4.3)$$

During anaerobic respiration of sulfates, SRBs produce large amounts of gaseous hydrogen sulfide (H_2S) which react easily in the water medium with heavy metal cations forming fairly insoluble metallic sulfides (Eq. 4.4):

$$Me^{2+} + H_2S \rightarrow MeS + 2H^+ \,(Me^{2+}\text{ - metal cation}). \qquad (4.4)$$

SRBs are of great economic importance especially in the oil industry. They are ubiquitous in oil-bearing shale and strata and therefore play an important economic role in many aspects of oil technology. They are:

1. Responsible for extensive corrosion of drilling and pumping machinery and storage tanks
2. Contaminate resulting crude oil and thereby increase undesirably the sulfur content of the oil through the H_2S which they release into it
3. Important in secondary oil recovery processes, where bacterial growth in injection waters can plug machinery used in these processes
4. Speculated to play a role in biogenesis of oil hydrocarbons

For all of these reasons, SRB are of vital importance in petroleum producing and processing industries.

Apart from the above, SRB are responsible for the corrosion of buried tanks and tanks made of iron; in some industries, such as the paper industry, they cause undesirable blackening of paper due to iron sulfides in the processing water.

In nature, sulfur circulates permanently because it is continuously oxidized or reduced by chemical or biological processes. In such a biogeochemical sulfur cycle (Fig. 4.16), the biological transformations may have either assimilatory or dissimilatory metabolic functions. SRB play an important in this cycle. Figure 4.16 shows the global sulfur cycle, including biological and nonbiological activities. The biological component of sulfur transformation is given in Fig. 4.17. Most plants, fungi, and bacteria are capable of performing an assimilatory reduction of sulfate to sulfide which is necessary for the biosynthesis of sulfur containing cell compounds. On the other hand, the energy producing dissimilatory sulfur metabolism is restricted to a few groups of bacteria. The bacteria which participate in the dissimilatory section of the biological sulfur cycle are collectively known as the sulfuretum.

These groups include:

(a) Anaerobic dissimilatory sulfate reducers (*Desulfovibrio*, *Desulfotomaculum*, *Desulfomonas*)
(b) Anaerobic dissimilatory sulfur reducers (*Desulfuromonas*, *Beggiatoa*)
(c) Anaerobic phototrophic sulfur oxidisers (some cyanobacteria and most anoxygenic phototrophic bacteria)
(d) Anaerobic chemotrophic sulfur oxidisers (*Thiobacillus denitrificans*, *Thiomicrospira denitrificans*)

Iron Bacteria

Iron bacteria are chemoautotrophs which derive energy by oxidizing dissolved ferrous iron, and sometimes manganese and aluminum. The resulting ferric oxide is insoluble, and appears as brown gelatinous slime that will stain plumbing fixtures, and clothing or utensils washed with the water carrying the oxide.

$$Fe^{2+} + 2H^+ + 0.5O_2 \Rightarrow Fe^{3+} + 0.5H_2O.$$

Iron bacteria grow in waters containing as low as 0.1 mg/l of iron. They produce the brownish scale that forms inside the tanks of flush toilets. They complete the oxidation of partially oxidized iron compounds and are able to couple the energy produced to the synthesis of carbohydrate.

Many different bacteria can be involved in producing oxidized iron seen as "rusty" sediments in water. The true iron bacteria are those whose metabolism has been described above. The genera involved are *Leptothrix,*, *Clonothrix, and Gallionella* and *Sphaerotilus.*They are usually stalked, filamentous, and difficult or impossible to cultivate. They are sheathed and the outer portion of the sheaths is covered with slime in which oxides of iron are deposited giving them the colors ranging from red to brown. This sheath makes them somewhat resistant to disinfectants.

Typical symptoms of iron bacterial growths in water supplies are:

(a) Discoloration of the waters (yellow to rust-red or brown)
(b) Reduction in flow rates through the system caused by coatings of iron bacteria inside the pipes
(c) Development of thick red or brown coatings on the sides of reservoirs, tanks, and cisterns; sometimes, sloughing off to form either fluffy specks in the water or gelatinous clumps of red to brown filamentous growths
(d) Rapid Clogging of Filter screens
(e) Heavy surface and sedimented growths of a red or brown color sometimes iridescent (ochre) in water

Iron bacteria do not cause disease and their nuisance value is mainly esthetic. They cause economic loss due to stained porcelain fixtures, fouled laundry, etc.

Iron bacteria are not active at temperatures of about 5°C or lower and they require water with iron content of at least 0.2 mg/l. They thrive in situations where there is good aeration, some source of nutrition, and some heat such as provided by water pumps, and a regular supply of water with dissolved iron. They are susceptible to ultraviolet of the sun and hence are found deep in the ground or hidden in pipes.

Heavy growths of iron bacteria form a substrate for other bacteria which may then degrade these materials anaerobically to form acidic products and hydrogen sulfide. The growth of iron bacteria can controlled through the use of chlorine.

It should be pointed out that passing that "rust" is not always solely due to bacterial activity but could be due to physicochemical reactions, especially where the geological formations contain iron oxides in the form of different iron minerals: Siderite (iron carbonate), pyrite or greigite (iron sulfide) and hematite (iron oxide or hydroxide). Ground water is low in oxygen and has pH near neutrality. The dissolved iron oxides can rise to as high as 5 mg/l under these conditions. When the water is pumped from underground, it is exposed to air and the dissolved oxides are quickly oxidized and sediment as fine rusty colored powder. Oxidizing agents such as chlorine and potassium permanganate accelerate the oxidation of the oxides and deposition of rust.

During water purification, the aeration of the raw also hastens the deposition of the oxides. Manganese oxides are frequently common in waters with iron oxides. They form black deposit when oxidized.

4.1.5 Archae

4.1.5.1 General Properties of Archaea

Like the Domain Bacteria, the Domain Archaea consist of single-celled organisms lacking nuclear membranes, and are therefore prokaryotes. A *single* organism from this domain is called an *archaeon*, just as a single member in the Domain Bacteria is a bacterium. As seen in Table 4.1 the properties of Archaea make them closer, evolutionarily, to Eukaryotes than they are to Bacteria. Thus their genetic transcription and translation do not show many typical bacterial features, and are in many aspects similar to those of eukaryotes. Many archaeal tRNA and rRNA genes harbor unique archaeal introns which are neither like eukaryotic introns, nor like bacterial introns. Several other characteristics also set the Archaea apart.

1. With the exception of one group of methanogens, Archaea lack a peptidoglycan wall. Even in this case, the peptidoglycan is very different from the type found in bacteria.
2. Archaeans also have flagella that are notably different in composition and development from the superficially similar flagella of bacteria. Flagella from both domains consist of filaments extending outside of the cell, and rotate to propel the cell. Recent studies show that there are many detailed differences between the archaeal and bacterial flagella:
   (a) Bacterial flagella are motorized by a flow of H^+ ions, wheras archaeal flagella move by the action of ATP.
   (b) Bacterial cells often have many flagellar filaments, each of which rotates independently; the archaeal

flagellum is composed of a bundle of many filaments that rotate as a single assembly.

(c) Bacterial flagella grow by the addition of flagellin (the protein in the flagella) subunits at the tip; archaeal flagella grow by the addition of subunits to the base.

(d) Bacterial flagella are thicker than archaeal flagella, and have a hole through which flagellin flows to be added at the tip, whereas archeal flagella are too thin for such a hole.

Many Archaea are inhabitants of aquatic environments, both marine and freshwater.

4.1.5.2 Taxonomic Groups Among Archeae

Archaea are divided into two main groups based on rRNA trees, the. Two other groups have recently been tentatively added: *Koracheota* and *Nanoarheota*. The discussion will be on the first two, and better known, groups.

Euryarchaeota

Members of this group can be arranged as follows:

1. *Extremely halohilic Archaea*: Members of this group survive in hypersaline environments, high levels of salt, such as are found in Great Salt Lake in Utah, and the Dead Sea. All are known as extremely Halophilic Archaea stain Gram negative. There are ten genera and 20 species of extreme halophiles, five of these genera contain only one species each: *Halobacterium*; *Halobaculum*; *Natrosobacterium*; Natrialba; Natrosomonas. The other genera are: *Natrarococcus* (two species); *Haloarcula* (two species); *Halococcus* (two species); *Haloferax* (four species); *Halorubrum* (five species). The key genera in this group are *Halobacterium,*, *Haloferax,* and *Natronobacterium.*
2. *Methane producing Archaea*: Nearly half of the known species of Archaea are unique in being capable of producing methane energy from selected low molecular weight carbon compounds and hydrogen as part of their normal biochemical pathways. Methanogens are anaerobic and are the most common and widely dispersed of the Archaea being found in anoxic sediments and swamps, lakes, marshes, paddy fields, landfills, hydrothermal vents, and sewage works as well as in the rumen of cattle, sheep, and camels, the cecae of horses and rabbits, the large intestine of dogs and humans, and in the hindgut of insects such as termites and cockroaches.

   In their natural habitats, methanogens depend on substrate supply from associated anaerobic microbial communities or geological sources, and depending on the substrates they utilize, three types of methanogenic pathways are recognized (see Fig. 4.18):

   (a) *Hydrogenotrophic methanogens* which grow with hydrogen (H_2) as the electron donor and carbon dioxide (CO_2) as the electron acceptor. Some hydrogenotrophs also use formate, which is the source of both CO_2 and H_2.

   (b) *Acetoclastic methanogens* which cleave acetate into a methyl and a carbonyl group. Oxidation of the carbonyl group into CO_2 provides potential for reduction of the methyl group into CH_4.

   (c) *Methylotrophic methanogens* grow on methylated compounds such as methanol, methylamines, and methyl sulfides, which act as both electron donor and acceptor or are reduced with H_2.

   The important genera among methane producing Archaea are *Methanobacterium*, *Methanosarcina* and *Methanocaldococcus*. Methanogens utilize a wide variety of substrates for producing methane. These include CO_2, alcohols, methyl substrates, methanol (CH_3OH), methylamine (CH_3NH^{3+}), and trimethylamine ($(CH_3)_3NH^+$) and acetic compounds such as acetate (CH_3COO^-) and pyruvate.
3. *Thermophilic and Extremely Acidophilic* (*Thermoplasmatales*): This is a small group of extreme acidophilic organisms. They containing four species in two genera, they are unusual in their ability to tolerate acid conditions. The two *Picrophilus* species are the most acidophilic organisms known. They have an optimal pH requirement of 0.7, can still grow at a pH of −0.06 and die at pH values of less than 4.0. Both *Picrophilus* species were found in acid solfatoras in Japan. Solfatoras are craters, often near volcanoes, spewing out steam, and gases such as CO_2, SO_2, and HCl. When sulfurous gases are spewed out from such craters they are solfatoras (from the Italian for sulfur). The two species of *Thermoplasma* grow optimally at pH 2.0. *Thermoplasma* spp. are also very unusual in that they do not have a cell wall. *T. volcanium* has been isolated from a number of solfatoras around the world. The cell membrane of *Thermoplasma* is composed of a lipopolysaccharide-like compound consisting of lipid with mannose and glucose units and called a lipoglycan. Examples of this group are Thermoplasma and Ferroplasma. These Archae lack cellwalls and in this regard are like Mycoplasmas. They not only

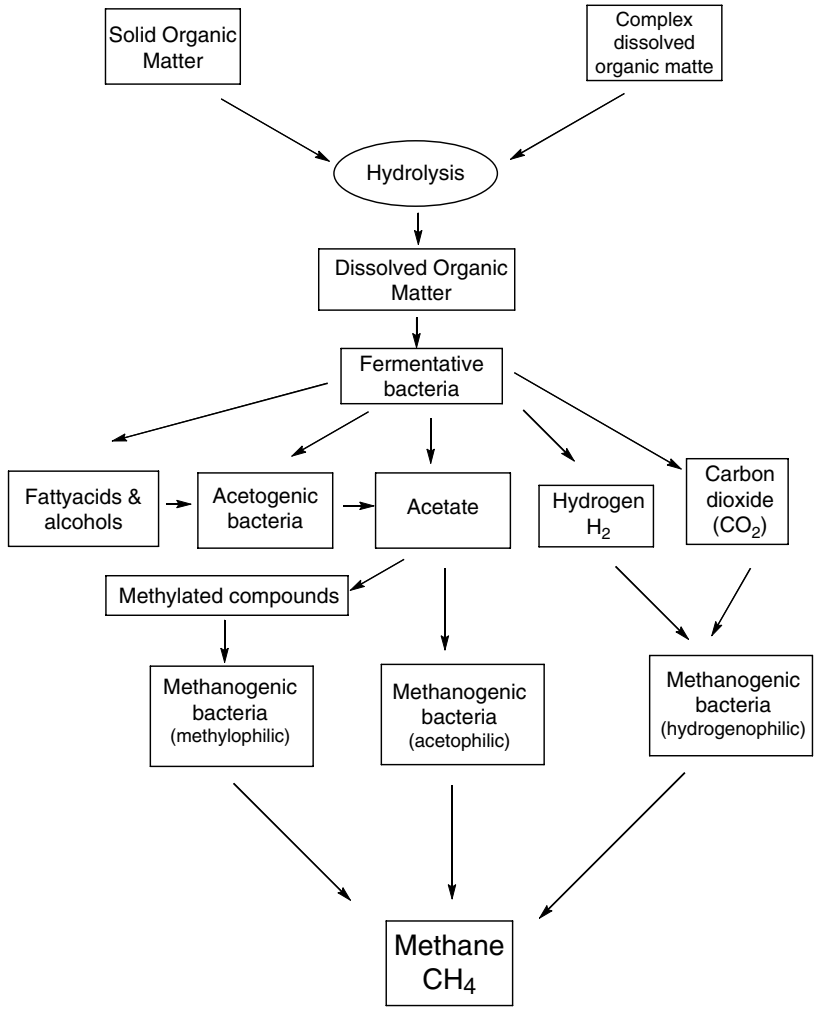


Fig. 4.18 Substrates and bacterial groups involved in methane production (After Christen and Kjelsen 1989)

survive without cellwalls but they also survive high temperatures and low acid conditions. For these conditions, these organisms have special polysaccaride structures in their cell membranes, a lipopolyssacharide.

4. *Hyperthermophilic Archae*: Well-known members of this group are *Thermococcus*, *Pyrococcus*, and *Methanopyrus.* Members of this group have optimal temperatures of 80°C and many grow at temperatures higher than that of boiling water. Thus *Thermococcus* and *Pyrococcus* (cocci with a tuft of flagella on one side) grow at between 70°c and 106°C with an optimum at 100°C (*Pyrococcus*). Proteins, starch or maltose are oxidized as electron donors and S^0 is the terminal acceptor and is reduced to H_2S.

Crenarchaeota

Crenarchaeota has the distinction of including microbial species with the highest known growth temperatures of any organisms. As a rule, they grow best between 80°C and 100°C and several species will not grow below 80°C. Several species also prefer to live under very acidic conditions in dilute solutions of hot sulfuric acid. Approximately 15 genera are known, and most of the hyperthermophilic species have been isolated from marine or terrestrial volcanic environments, such as hot springs and shallow or deep-sea hydrothermal vents. Recent analyses of genetic sequences obtained directly from environmental samples, however, indicate the existence of low temperature Crenarchaeota, which have not yet been cultivated. The most spectacular feature of the Crenarchaeota, however, is their tolerance

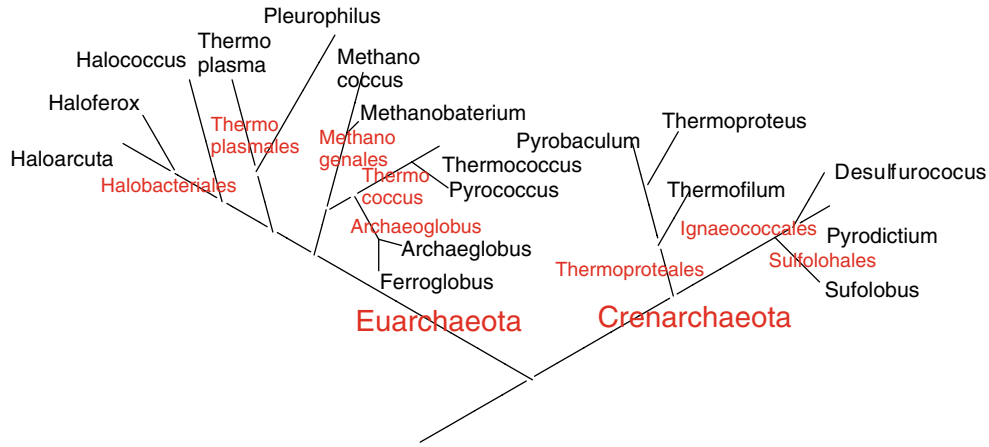


Fig. 4.19 Phylogenetic tree of the Archae (Modified from Ciccarelli et al. 2006)

Note: The genera of Archeae are given above. They are placed in the two phyla of Archeae which have been cultivated, Euarchaeota and Crenarchaeota. A third phylum, the **Korarcheota** are only known from their DNA sequences and have not yet been cultivated. The notations in *red* are orders in which the genera are grouped. Thus the halophilic archeae are grouped among the Halobacteriales the methane producers are in Methagenales

of, and even preference for, extremes of acidity and temperature. While many prefer neutral to slightly acidic pH ranges, members of the Crenarchaeal order Sulfolobales flourish at pH 1–2 and die above pH 7. Optimum growth temperatures range from 75°C to 105°C and the maximum temperature of growth can be as high as 113°C (*Pyrobolus*). Most species are unable to grow below 70°C, although they can survive for long periods at lower temperatures. Crenarchaeota contains representations of organisms which live in a wide variety of environments including terrestrial environments (hot springs, geothermal power plants) or in marine (submarine hot vents, deep oil wells, marine smokers up to 400°C). Some exist in environments of over 100°C, while others live at ice cold conditions. For substrates, they utilize a wide range of gases: CO_2, CO, CH_4, S_2O_3, N_2, NH_4 (Fig. 4.19).

(a) *Hyperthermophiles in underwater volcanic environments*
Temperatures as high as 100°C occur around terrestrial volcanoes and *Sulfolobus* and *Thermoproteus*, both hyperthermophiles, have been isolated from such environments.

(b) *Hyperthermophiles in land volcanic environments*
Archae with the highest optimum temperature of growth known occur in underwater vents and near underwater volcanoes. *Pyrodictium* sp. and *Pyrolobus* sp. have optimum temperatures of growth of 100°C and 106°C respectively and are found in such environments. *Desulforococcus* (90°C, optimum) and *Staphylothermus* (95°C, optimum) are also found in that environment.

4.1.6 Microbial Taxonomic Groups Among Eucharia

The Domain Eukarya includes plants, animals, algae, fungi, and protozoa. The last three are regarded as microorganisms, although some of them are quite large. In Fig. 4.20, green algae, brown algae, red algae, and diatoms are Algae. Diplomonads, Trichomonads, Ciliates, Flagellates and Slime molds are Protozoa.

4.1.6.1 Protozoa

Protozoa are thought to be the evolutionary ancestors of all multi-cellular organisms, including plants, fungi, and animals. The basis for this assumption is that Protozoa contain members which like plants are phototrophic, as well as members which like animals and fungi are heterotrophic. Furthermore Protozoa contain species with intermediate (mixotrophic) trophic capabilities, i.e., they have the capability for both auto- and heterotrophic existence. Thus, many dinoflagellates are auxotrophic because they take up vitamins produced by other organisms; on the other hand, other Protozoa such as some euglenoids alter from one form of trophic existence system to another.

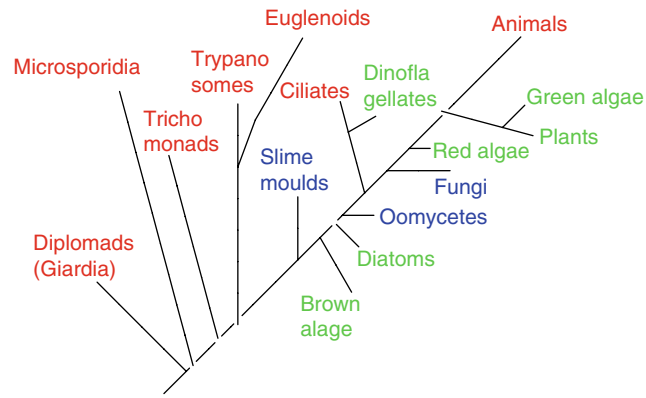


Fig. 4.20 Phylogenetic tree among the Eukarya (Redrawn from Ciccarelli et al. 2006)

Protozoa are classified in many ways, primarily on morphological characteristics, and the one adopted here groups them into five: Mastigophora, Sarcodina, Ciliata, Sporozoa, and Suctoria.

1. *Flagellata* (*Mastigophora*)
   These possess flagella and are subdivided into "phytoflagellata" and "zooflagellata," depending on whether they are plant-like (with chlorophyll) or animal-like (without chlorophyll). They usually multiply by longitudinal binary fission. Many flagellates are able to feed autotrophically as well as heterotrophically, and are important primary producers in lakes and oceans; yet, they can also feed like animals, ingesting or absorbing food synthesized by other organisms.

   Many are free-living, but some are parasitic. Examples of parasitic Mastigophora are *Trypanosoma gambiense* and *T. rhodesiense* which cause African sleeping sickness and is transmitted by tsetse flies. *T. cruzi* is the cause of Chagas' disease, prevalent in South and Central America, which affects the nervous system and heart; it is transmitted by the bite of assassin bugs. *Giardiasis* is caused by the mastigophoran *Giardia lamblia.*
2. *Rhizopoda* (*Sarcodina*)
   These Protozoa use pseudopodia (false feet) for locomotion and for catching preys. Members of the group Sarcodina move by pseudopodia; although, flagella may be present in the reproductive stages. Cytoplasmic streaming assists movement. Asexual reproduction occurs by fission of the cell.

   Sarcodina includes two marine groups known as foraminiferans and radiolarians. Both groups were present on earth when the oil fields were in formative stages, and marine geologists use them as potential markers for oil fields. Some amoebae live in shells from which the pseudopodia are extruded. Some members of the group such as *Entamoeba histolytica*, are pathogenic, causing amoebic dysentery in humans. This organism can cause painful lesions of the intestine and is contracted in polluted water (Fig. 4.21).
3. *Ciliata*
   Ciliates possess cilia (short and highly coordinated flagellae), a somatic (macro) nucleus, and genetic (micro) nucleus, and a contractile vacuole is usually present. They move by means of cilia. Conjugation may be used for sexual reproduction and binary fission also occurs. The distinctive rows of cilia vibrate in synchrony and propel the organism in one direction. One of the best known members of the group is *Paramecium*; another of the free-living members of this group, is *Tetrahymena.*

   Ciliates form an extremely large group are distinguished by the possession of cilia, two different types of nuclei and transverse fission of the organism when it divides, unlike flagellates and sarcodina which divide longitudinally.
4. *Sporozoa*
   Members of the group *Sporozoa* form spores at one stage in their life cycle. Sporozoa are endoparasites which have spores. Most of them spend at least part of their life-cycle inside a host cell. Reproduction is a complex phenomenon in this group. Members of the group display no means of locomotion in the adult form. Their motile stages move by bending, creeping, and gliding and usually have an apical complex at their anterior end which help them penetrate their hosts. The group includes *Plasmodium*, the agent of malaria, *Toxoplasma*, the agent of toxoplasmosis, and *Pneumocystis carinii,* the cause of a serious pneumonia in AIDS patients.

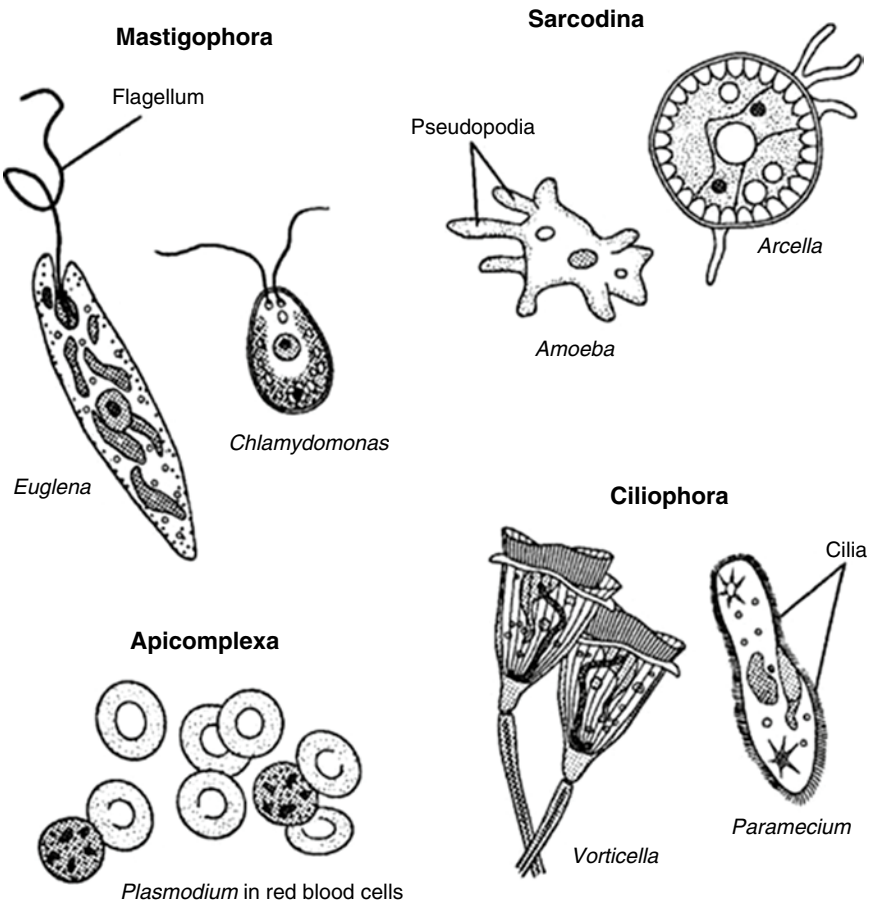


Fig. 4.21 Protozoan groups. Illustrations of some Protozoa (From http://www.cliffsnotes.com/study_guide/topicArticleId-8524,articleId-8461.html; Anonymous 2010b. With permission)

5. *Suctoria*
   The juvenile forms are ciliated and motile, while the adult forms are sessile and capture food by tentacles. They feed by extracellular digestion and lack cilia in the adult phase. The adult have structures called haptocysts at the tip which attach to the prey. The prey's cytoplasm is then sucked directly into a food vacuole inside the cell, where its contents are digested and absorbed. Most suctoria are around 15–30 μm in size, with a non-contractile stalk and often a shell. Suctoria reproduce primarily by budding, producing swarmers which lack both tentacles and stalks but have cilia. Once the swarmers (motile young) have found a place to attach themselves, they quickly develop stalks and tentacles and lose their cilia. Because of the presence of cilia in the young of suctoria, some authors group the suctoria among ciliates.

   Suctoria are found in both freshwater and marine environments, and some which live on the surface of aquatic animals, and typically feed on ciliates. Some marine species form symbiotic relationships with crustaceans and even some fish. One species, *Ephelota gemmipara* lives on the external parasite of salmon, *Lepeophtheirus salmonis* (salmon louse).

4.1.6.2 Fungi

Fungi are eukaryotic microorganisms which

(a) Are non-photosynthetic and hence do not contain chlorophyll
(b) Contain chitin and/or cellulose in their cell walls
(c) Are usually filamentous (called molds), but they may be unicellular (called yeasts)
(d) Reproduce asexually with spores

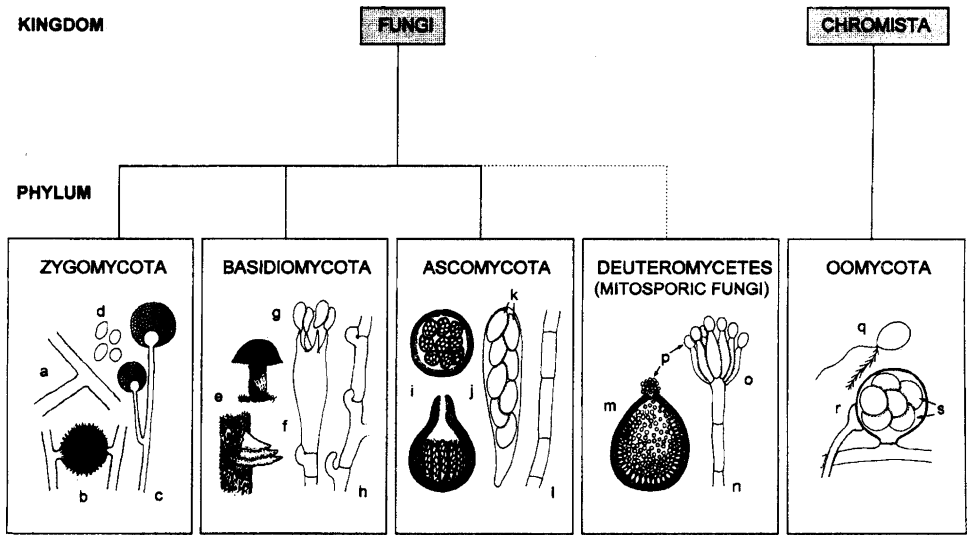


Fig. 4.22 Diagnostic features of fungi (From Guarro et al. 1999)
Zygomycota (Phycomycetes): (a) coenocytic hypha; (b) zygospore; (c) sporangiophore; (d) sporangiospores.
Basidiomycota (Basidiomycetes): (e) basidiomate; (f) basidium; (g) basidiospore; (h) hypha with clamp connections.
Ascomycota (Ascomycetes): (i) ascommata; (j) ascus; (k) ascospores; (l) septate hyphae.
Deuteromycota (Deuteromycetes): (m) pycnidium; (n) conidiophore; (o) conidiogenous cells; (p) conidia.
Oomycota (regarded as aquatic Phycomycetes) : (q) zoospore; (r) gametangia; (s) oospores

Taxonomy of Fungi

The classification of the fungi is based mainly on morphology of the hyphae, the structures housing the sexual structures, or the structure to which the sexual spores are attached (Samson and Pitt 1989; Guarro et al. 1999). The principal diagnostic characteristics are shown in Fig. 4.22 and are as follows:

(a) *Septation of the hyphae*
The septation, or lack thereof, of the hyphae is important in classifying fungi. Non-septate or coenocytic hyphae are found in Phycomycetes (Zygomycota). All other fungal groups have septate hyphae. Examples of Phycomycetes are *Mucor* spp. and *Rhizopus* spp., the bread mold.

(b) *The nature of the asexual spores of aquatic Phycomycetes*
Aquatic fungi are found among Phycomycetes. The asexual cells of many aquatic Phycomycetes are motile and are flagellated and help in the identification of the organisms (see Figs. 4.22 and 4.23). Many aquatic Phycomycetes, classified as Oomycetes, by some authors are pathogens of plants and fish. *Phytphthora infestans* which caused the famous potato blight and subsequent famine in Ireland which led to massive Irish immigration to the US belong to this group. Others are *Plasmopara viticola* (the cause of downy mildew of grapes), *Plasmopara halstedii* (sunflower downy mildew), and *Saprolegniales* spp., or water molds, which cause diseases of fish and other aquatic vertebrates. Some authors argue that the Oomycetes are so different from other fungi (so-called true fungi) that they should not be classified with them. The majority of authors classify them with the Phycomycetes, their peculiarities notwithstanding (see Table 4.8).

(c) *The presence of asci*
An ascus or sac (*plural*, asci) which contains ascosposcores (sexual spores typically eight in number housed in an ascus) is diagnostic of *Ascomycetes* (Ascomycota)**.** The trivial name of this group of fungi is sac fungi.

(d) *The presence of Basidiomycetes*
The presence of basidiospores, typically four in number attached to a basidium, (a club-like structure) identifies Basidiomycetes. Some of the best known *Basidiomycetes* (Basidiomycota) are mushrooms. A microscopic examination of the "gills" on the underside of the mushrooms reveals the basidia carrying the basidiospores.

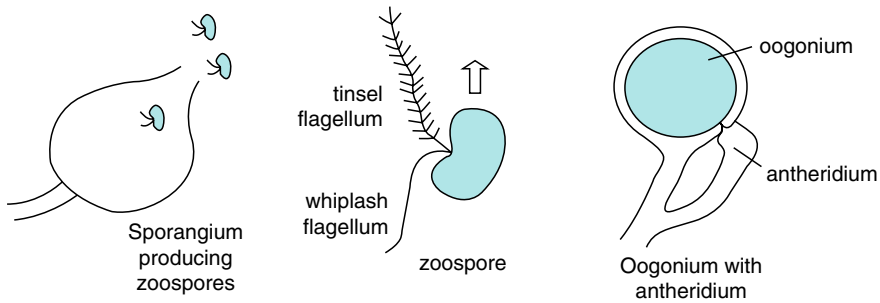


Fig. 4.23 Reproductive structures in aquatic fungi (From Rossman and Palm 2006. With permission)

Table 4.8 Properties of oomycetes and other ("true") fungi (Modified from Rossman and Palm 2006. With permission)

Character	Oomycota	True Fungi
Sexual reproduction	Heterogametangia. Fertilization of oospheres by nuclei from antheridia forming oospores	Oospores not produced; sexual reproduction results in zygospores, ascospores, or basidiospores
Nuclear state of vegetative mycelium	Diploid	Haploid or dikaryotic
Cell wall composition	Beta glucans, cellulose	Chitin. Cellulose rarely present
Type of flagella on zoospores, if produced	Heterokont, of two types, one whiplash, directed posteriorly, the other fibrous, ciliated, directed anteriorly	If flagellum produced, usually of only one type: posterior, whiplash
Mitochondria	With tubular cristae	With flattened cristae

(e) *Deutromycetes* (*Deuteromycota*) *or fungi imperfecti*
These are fungi whose perfect or sexual stages have not been discovered. Some of the best examples are *Penicillin* spp. (with broom-like structures) and *Aspergillus* spp. (with club-like structures).

4.1.6.3 Algae

Algae are photosynthetic eukaryotic organisms which lack the structures of vascular plants. Many authors classify them as microorganisms, but they are highly variable in size and range from microscopic sizes to the brown algae which could be up to 70 m long (Trainor 1978; Sze 1986) (Fig. 4.24).

Taxonomy of Algae

The classification of the algae is based on the following:

(a) *Pigmentation*
The various kinds of pigments in the algae, as well as the overall color of the alga, are used in classifying the organisms. All of the groups contain the following pigments which are soluble in organic solvents: Chlorophylls and several carotenoids, which include carotenes and xanthophylls. Chlorophylls "a" and "b," alpha or beta carotene, and some xanthophylls are common. The water soluble phycobiliproteins (phycobilins) are found in blue-green algae, red algae, and a small group of flagellates.

(b) *The reserve compound*
Reserve food material is usually stored within the cell and frequently within the plastid in which photosynthesis occurred. Starch, starchlike compounds, fats, or oils are the most common forms.

(c) *The nature of the zoospores*
Some organisms are motile during much of their lives, whereas other genera lack motility, or any motile reproductive stages. Adult algae are usually nonmotile; often, however, some reproductive stages (zoospores) are motile. The overall shape of the zoospores, the shape, number, and the insertion position of the flagella, and the presence or absence of hairs on the flagella are diagnostic (Fig. 4.24; Table 4.9).

(d) *Wall composition*
The cell wall may be a simple outer covering around the protoplast or an elaborately ornamented structure. The materials found in algal walls are cellulose, xylans, mannans, sulfated polysaccharides, alginic acid, protein, silicon dioxide, and calcium carbonate

(e) *Gross morphology of the alga*
The overall shape of the alga is diagnostic.

The various groups of algae are given in the Table 4.9.

Fig. 4.24 Illustrations of some algae (All items in the table reproduced with permission) a) Red algae are red because of the presence of the pigment phycoerythrin; this pigment reflects red light and absorbs blue light. Because blue light penetrates water to a greater depth than light of longer wavelengths, these pigments allow red algae to photosynthesize and live at somewhat greater depths than most other algae. The picture of the red alga, Dichotomaria marginata, shown here was taken and kindly supplied by Keoki Stender, University of Hawaii. b) Sargassum fluitans. Sargassum seaweed, Gulf Weed (brown alga). This is a major component of the algae in the Sargasso Sea in the pelagic Atlantic. It has long, serrated fronds with a distinctive mid-rib, and smooth berry-like spherical gas-filled bladders, pneumatocysts, which assist the floatation of the alga.The photo of Sargassum above was kindly supplied by the South Carolia Department of Natural Resource, courtesy of H. Scott Meister. c) Synura spp, a member of the Chrysophyceae (golden or golden-brown algae on account of their content of fucoxanthin which in the presence of chlorophyll makes them look brown or golden brown), forms swimming colonies from a variable number of cells joined together at their posterior ends in a spherical or elongated cluster. Synura is important because it gives drinking water a bitter taste and a "fishy" cod liver oil type of odour. Synura is freshwater; some marine members such as Olisthodiscus luteus, produce neurotoxins which may kill aquatic fauna and may affect humans through eating shell fish raw. Credit: Dr Graham Matthews, graham@gpmatthews.nildram.co.uk (http://www.gpmatthews.nildram.co.uk/microscopes/pondlife_plants01.html). Dr Graham Mathews is also Hon Secretary of the Secretary of the Quekett Microscopical Club) d) Bacillaropyceae (Diatoms) are one of the largest and ecologically most significant groups of organisms on Earth. Diatoms are microscopic algae which are easily recognizable because of their unique cell structure, silicified cell wall and life cycle. Diatoms are found anywhere there is water and light: in oceans, lakes and rivers; marshes, fens and bogs; damp moss and rock faces. They are an important part of the food chain in aquatic environments, especially in nutrient-rich areas of the world's oceans, where they occur in abundance.Photograph of diatoms kindly supplied by Dr David Carling) e) Dinoflagellates are minute marine unicellular algae with diverse morphology, the largest, Noctiluca, being as large as 2 mm in diameter!. Many are photosynthetic, while some are parasites of fish. In temperate climates they form blooms in summer months which may be golden or red. Their blooms produce neurotoxins marine animals eating and humans who consume them raw (such as shellfish)

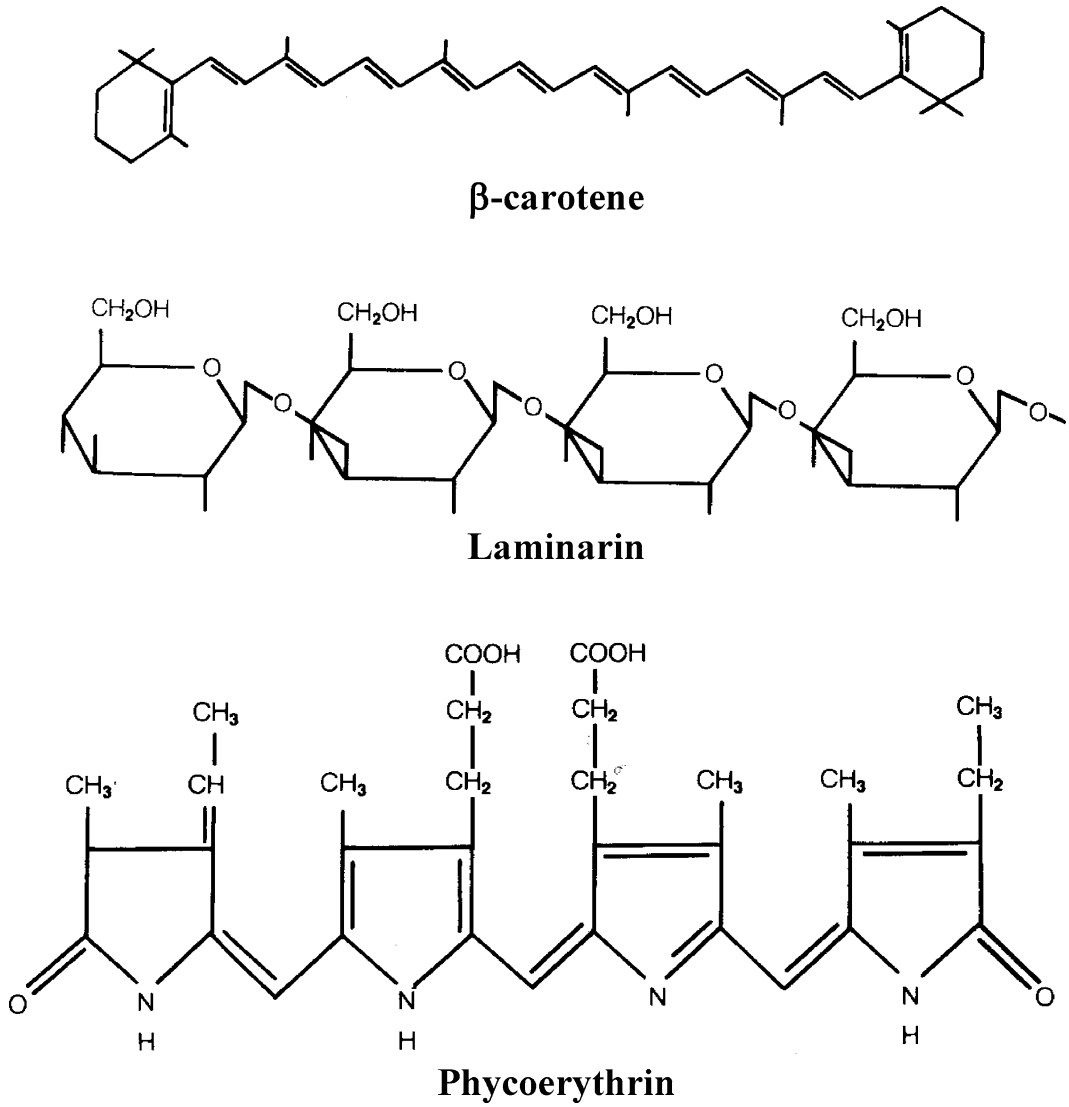


Fig. 4.25 Some pigments and storage material of algae

4.1.7 Viruses

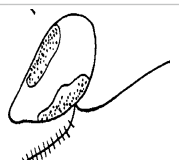
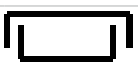
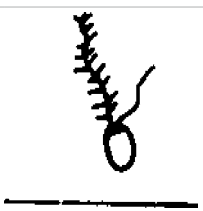
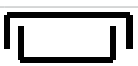
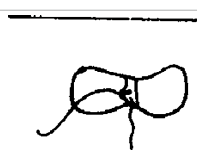
Until recently, viruses were not thought to be abundant or important in the aquatic environment. We now know that they are not only abundant, but that they profoundly influence the ecology and food status of the aquatic environment including seas and oceans.

Viruses are lifeless crystals of nucleic acid which are able to grow and reproduce only in living cells. They differ from cells in the following ways, and also have the following properties:

1. Whereas cells contain both DNA and RNA, viruses contain either DNA or RNA, never both.
2. Viruses have a nucleic acid inner core (the genome) and outer protein cover, the capsid (see Fig. 4.26).
3. Viruses enter only susceptible cells: thus there are viruses which will attack only plants while some will attack only animals. Even among plants and animals, some viruses will attack some members and not the others.
4. All living things are attacked by viruses, including the microorganisms: Bacteria, fungi, algae, and protozoa. Viruses attacking bacteria and fungi are bacteriophages and mycophages, respectively.

Viruses used to be classified on their diseases they cause and their sizes and shapes, but these criteria have

Table 4.9 The various algal groups (Compiled from Sze 1986 and Trainor 1978)

S. No.	Group	Pigments	Storage	Zoospores	Walls,	Morphology	Example	Habitat
1.	Chlorophyceae (Green algae)	Chlorophyll a,b, Xanthophyll	Starch	Variale, when present	Cellulose, chitin, calcium carbonate	Unicellular to multicellular	Spirogyra, Chlamydomonas	Freshwater, oceans, soils
2.	Rhodophyceae (Red algae)	Chlorophyll a,d Carotene a, b Phycobilins (masks other pigments)	Floridean starch (like glycogen)	None	Cellulose	Multicellular: large, up to 5 m long	*Gelidium* (agar, microbial cultivation) and *Chondrus* (carageenan – food thickening)	Few freshwater, mostly marine:warmer waters, especially along coastlines
3.	Phaemyceae (Brown algae)	Chlorophyll a,c Carotene a, b Xanthophylls	Laminarin, mannitol, fat		Alginic acid, cellulose	Multicellular: large, up to 5 m long	Ectocarpus	Mostly marine in north temperate regions
4.	Bacillariophyceae (Diatoms)	Chlorophyll a,c Xanthophylls	Glucans, Oil	No moving member at any stage	 Celluose, pectin in above structure	Unicels, colonial or filametous	*Tabelaria* spp.(freshwater)	Marine and freshwater
5.	Xanthophyceae (Yellow green algae)	Chlorophyll a,c Carotene a, b Xanthophylls	Chrysolaminarin, Oil		 Celluose, pectin in above structure	Unicells, colonies, branched and unbranched filaments	*Vaucheria* sp.	Mostly temperate freshwater
6.	Chrysophyceae (Golden algae)	Chlorophyll c Carotene a, b Xanthophylls	Chrysolaminarin	Adults nonmotile or motile with a flagellum	Naked or cellulose	Typically Unicellular; few colonies, branched and unbranched filaments	*Synura* sp. (imparts fish odor to water)	Cold freshwater in northern hemisphere
7.	Dinophyceae (Dinoflagellates; dark-brown algae)	Chlorophyll c Carotene b Xanthophylls	Starch or oils		Often only cell membrane; sometimes a pellicle Girdle in which one flagellum lodged; other flagellum trails	Motile adult flagellates, colonies or filamentous		Freshwater and marine

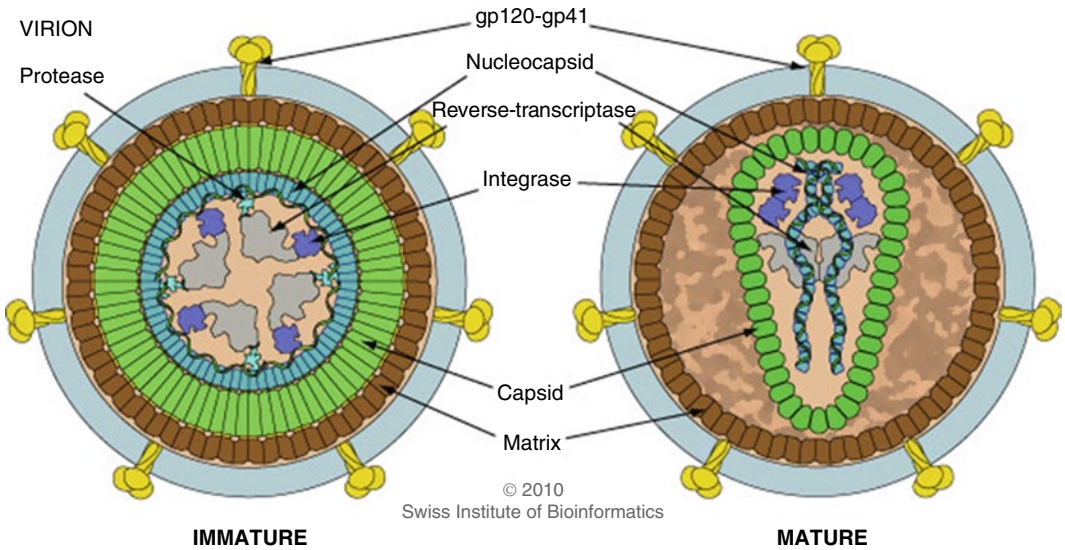


Fig. 4.26 Structure of HIV 1 virus (Reproduced with permission from the Swiss Institute of Bioinformatics [SIB]; Anonymous 2010c)

Note that the HIV 1 virus has an envelope (matrix in the diagram above). Not all viruses have envelopes; those which do not are said to be *naked* (see text). The gp structures are glycoprotein. (Retroviral) integrase is an enzyme produced by a retrovirus that enables its genetic material to be integrated into the DNA of the infected cell. Note also the reverse transcriptase (produced by retroviruses such as HIV), a DNA polymerase enzyme that transcribes single-stranded RNA into double-stranded DNA

been abandoned owing to the small sizes of viruses and because the disease symptoms of different viruses were sometimes similar (Anonymous 2005; Sander 2007). The current classification of viruses is credited to David Baltimore, who won the Nobel Prize for his discovery of retroviviruses and reverse transcriptase. According to the Baltimore classification, viruses are grouped into seven based on their nucleic acid (DNA or RNA), strandedness (single-stranded or double stranded), and method of replication. The groups are numbered with Roman numerals thus:

Group I: double-stranded DNA viruses
Group II: single-stranded DNA viruses
Group III: double-stranded RNA viruses
Group IV: positive-sense single-stranded RNA viruses
Group V: negative-sense single-stranded RNA viruses
Group VI: reverse transcribing diploid single-stranded RNA viruses
Group VII: reverse transcribing circular double-stranded DNA viruses

Nomenclature of viruses: The nomenclature of viruses is based on a set of rules set up by the International Committee on the Taxonomy of Viruses (ICTV), which since the 1960s has been arranging viruses in these seven groups into taxonomic hierarchies.

The criteria for the taxonomic arrangements are:

1. *Morphology*
   (Helical, e.g., bacteriophage M13; icosohedral/polyhedral/cubic, e.g., poliovirus, enveloped – may have poyhedral (e.g., herpes simplex) or helical (e.g., influenza virus) capsids, complex, e.g., poxviruses)
2. *Nucleic acid type*,
3. Whether the virus is *naked or enveloped*
4. *Mode of replication*
5. *Host organisms*
6. The *type of disease* they cause

4.1.7.1 Viral Taxonomy and Nomenclature

Viral taxonomic nomenclature is modeled after that of cellular organisms. However viruses suffer from the absence of fossil record which will enable more phylogenetic relationships among the various groups. Consequently, the highest level in the viral taxonomic hierarchy is the order, thus:

Order (*-virales*)
Family (*-viridae*)
Subfamily (*-virinae*)
Genus (*-virus*)
Species (*-virus*)

Regarding nomenclature, the rules set up by the ICTV are as follows (Van Regenmortel 1999):

- The names of virus orders, families, subfamilies, genera, and species should be written in italics with the first letter capitalized.
- Other words are not capitalized unless they are proper nouns, e.g., *Tobacco mosaic virus*, *Poliovirus*, *Murray River encephalitis virus.*
- This format should only be used when official taxonomic entities are referred to - it is not possible to centrifuge the species, for example, *Poliovirus,* but it is possible to centrifuge poliovirus.
- Italics and capitalization are not used for vernacular forms (e.g., rhinoviruses, c.f. the genus *Rhinovirus*), for acronyms (e.g., HIV-1), nor for adjectival usage (e.g., poliovirus polymerase).

4.1.7.2 The Viral Groups

DNA Viruses (Groups I and II)

Group I: These are double-stranded DNA viruses and include such virus families as Herpesviridae (examples like HSV1 [oral herpes], HSV2 [genital herpes], VZV [chickenpox], EBV [Epstein–Barr virus], CMV [Cytomegalovirus]), Poxviridae (smallpox), and many tailed bacteriophages. The mimivirus is also placed into this group (see Table 4.10).

Group II: These viruses possess single-stranded DNA and include such virus families as Parvoviridae and the important bacteriophage M13 (see Table 4.11).

RNA Viruses (Groups III, IV and V)

Group III: These viruses possess double-stranded RNA genomes, e.g., rotavirus. These genomes are always segmented. Segmented virus genomes are those which are divided into two or more physically separate molecules of nucleic acid, all of which are then packaged into a single virus particle (see Table 4.12).

Group IV: These viruses possess positive-sense single-stranded RNA genomes. Many well known viruses are found in this group, including the picornaviruses (which is a family of viruses that includes well-known viruses like Hepatitis A virus, enteroviruses, rhinoviruses, poliovirus, and foot-and-mouth virus), SARS virus, hepatitis C virus, yellow fever virus, and rubella virus. Positive-sense viral RNA is identical to viral mRNA and thus can be immediately translated by the host cell (see Table 4.13).

Group V: These viruses possess negative-sense single-stranded RNA genomes. The deadly Ebola and Marburg viruses are well known members of this group, along with influenza virus, measles, mumps, and rabies. Negative-sense viral RNA is complementary to mRNA and thus must be converted to positive-sense RNA by an RNA polymerase before translation (see Table 4.14).

Reverse Transcribing Viruses (Groups VI and VII)

A *reverse transcribing virus* is any virus which replicates using reverse transcription, the formation of DNA from an RNA template. Both Group VI and Group VII viruses fall into this category. Group VI contains single-stranded RNA viruses which use a DNA intermediate to replicate, whereas Group VII contains double-stranded DNA viruses which use an RNA intermediate during genome replication. Thus a reverse transcriptase, also known as RNA-dependent DNA polymerase, is a DNA polymerase enzyme that transcribes single-stranded RNA into single-stranded DNA. Normal transcription involves the synthesis of RNA from DNA; hence, reverse transcription is the *reverse* of this.

Group VI: *RNA Reverse Transcribing Viruses* possess single-stranded RNA genomes and replicate using reverse transcriptase. The retroviruses are included in this group, of which HIV is a member. Members of Group VI use virally encoded reverse transcriptase, a RNA-dependent DNA polymerase, to produce DNA from the initial virion RNA genome. This DNA is often integrated into the host genome, as in the case of retroviruses and pseudoviruses, where it is replicated and transcribed by the host. Group VI includes the following in Table 4.15.

Group VII: *DNA Reverse Transcribing Viruses* possess double-stranded DNA genomes and replicate using reverse transcriptase. The hepatitis B virus can be found in this group. Group VII have DNA genomes contained within the invading virus particles. The DNA genome is transcribed into both mRNA, for use as a transcript in protein synthesis, and pre-genomic RNA, for use as the template during genome replication. Virally encoded reverse transcriptase uses the pre-genomic RNA as a template for the creation of genomic DNA. They are shown in Table 4.16.

The Structure of the DNA and RNA Viruses

The structures of the various viruses whether they are DNA or RNA are highly variable. The structure of the DNA viruses are shown in Table 4.17 and those of the RNA viruses are shown in Table 4.18.

Table 4.10 Group I: dsDNA Viruses (Reproduced from Van Regenmortel et al. 2005; http://www.microbiologybytes.com/virology/VirusGroups.html#VI; Anonymous 2005. With permission)

Order *Caudovirales* – tailed bacteriophages				
Family				
Subfamily		Genus	Type species	Hosts
Myoviridae		T4-like viruses	*Enterobacteria phage T4*	Bacteria
		P1-like viruses	*Enterobacteria phage P1*	Bacteria
		P2-like viruses	*Enterobacteria phage P2*	Bacteria
		Mu-like viruses	*Enterobacteria phage Mu*	Bacteria
		SP01-like viruses	*Bacillus phage SP01*	Bacteria
		ϕH-like viruses	*Halobacterium virus ϕH*	Bacteria
Podoviridae		T7-like viruses	*Enterobacteria phage T7*	Bacteria
		P22-like viruses	*Enterobacteria phage P22*	Bacteria
		ϕ29-like viruses	*Bacillus phage ϕ29*	Bacteria
		N4-like viruses	*Enterobacteria phage N4*	Bacteria
Siphoviridae		λ-like viruses	*Enterobacteria phage λ*	Bacteria
		T1-like viruses	*Enterobacteria phage T1*	Bacteria
		T5-like viruses	*Enterobacteria phage T5*	Bacteria
		L5-like viruses	*Mycobacterium phage L5*	Bacteria
		c2-like viruses	*Lactococcus phage c2*	Bacteria
		ψM1-like viruses	*Methanobacterium virus ψM1*	Bacteria
		ϕC31-like viruses	*Streptomyces phage ϕC31*	Bacteria
		N15-like viruses	*Enterobacteria phage N15*	Bacteria
Ascoviridae		*Ascovirus*	*Spodoptera frugiperda ascovirus*	Invertebrates
Adenoviridae		*Atadenovirus*	*Ovine adenovirus D*	Vertebrates
		Aviadenovirus	*Fowl adenovirus A*	Vertebrates
		Mastadenovirus	*Human adenovirus C*	Vertebrates
		Siadenovirus	*Frog adenovirus*	Vertebrates
Asfarviridae		*Asfivirus*	*African swine fever virus*	Vertebrates
Baculoviridae		*Nucleopolyhedrovirus*	*Autographa californica nucleopolyhedrovirus*	Invertebrates
		Granulovirus	*Cydia pomonella granulovirus*	Invertebrates
Corticoviridae		*Corticovirus*	*Alteromonas phage PM2*	Bacteria
Fuselloviridae		*Fusellovirus*	*Sulfolobus virus SSV1*	Archaea
Guttaviridae		*Guttavirus*	*Sulfolobus virus SNDV*	Archaea
Herpesviridae:		*Ictalurivirus*	*Ictalurid herpesvirus 1*	Vertebrates
	Alphaherpesvirinae	*Mardivirus*	*Gallid herpesvirus 2*	Vertebrates
		Simplexvirus	*Human herpesvirus 1*	Vertebrates
		Varicellovirus	*Human herpesvirus 3*	Vertebrates
		Iltovirus	*Gallid herpesvirus 1*	Vertebrates
	Betaherpesvirinae	*Cytomegalovirus*	*Human herpesvirus 5*	Vertebrates
		Muromegalovirus	*Murine herpesvirus 1*	Vertebrates
		Roseolovirus	*Human herpesvirus 6*	Vertebrates
	Gammaherpesvirinae	*Lymphocryptovirus*	*Human herpesvirus 4*	Vertebrates
		Rhadinovirus	*Simian herpesvirus 2*	Vertebrates
Iridoviridae		*Iridovirus*	*Invertebrate iridescent virus 6*	Invertebrates
		Chloriridovirus	*Invertebrate iridescent virus 3*	Invertebrates
		Ranavirus	*Frog virus 3*	Vertebrates
		Lymphocystivirus	*Lymphocystis disease virus 1*	Vertebrates
		Megalocytivirus	*Infectious spleen and kidney necrosis virus*	Vertebrates

(continued)

Table 4.10 (continued)

Order *Caudovirales* – tailed bacteriophages				
Family				
Subfamily		Genus	Type species	Hosts
Lipothrixviridae		*Alphalipothrixvirus*	*Thermoproteus virus 1*	Archaea
		Betalipothrixvirus	*Sulfolobus mislandicus filamentous virus*	Archaea
		Gammalipothrixvirus	*Acidianus filamentous virus 1*	Archaea
Nimaviridae		*Whispovirus*	*White spot syndrome virus 1*	Invertebrates
Mimivirus			*Acanthamoeba polyphaga mimivirus*	Protozoa, Vertebrates
Polyomaviridae		*Polyomavirus*	*Simian virus 40*	Vertebrates
Papillomaviridae		*Alphapapillomavirus*	*Human papillomavirus 32*	Vertebrates
		Betapapillomavirus	*Human papillomavirus 5*	Vertebrates
		Gammapapillomavirus	*Human papillomavirus 4*	Vertebrates
		Deltapapillomavirus	*European elk papillomavirus*	Vertebrates
		Epsilonpapillomavirus	*Bovin papillomavirus 5*	Vertebrates
		Zetapapillomavirus	*Equine papillomavirus 1*	Vertebrates
		Etapapillomavirus	*Fringilla coelebs papillomavirus*	Vertebrates
		Thetapapillomavirus	*Psittacus erithacus timneh papillomavirus*	Vertebrates
		Iotapapillomavirus	*Mastomys natalensis papillomavirus*	Vertebrates
		Kappapapillomavirus	*Cottontail rabbit papillomavirus*	Vertebrates
		Lambdapapillomavirus	*Canine oral papillomavirus*	Vertebrates
		Mupapillomavirus	*Human papillomavirus 1*	Vertebrates
		Nupapillomavirus	*Human papillomavirus 41*	Vertebrates
		Xipapillomavirus	*Bovine papillomavirus 3*	Vertebrates
		Omikronpapillomavirus	*Phocoena spinipinnis papillomavirus*	Vertebrates
		Pipapillomavirus	*Hamster oral papillomavirus*	Vertebrates
Phycodnaviridae		*Chlorovirus*	*Paramecium bursaria Chlorella virus 1*	Algae
		Prasinovirus	*Micromonas pusilla virus SP1*	Algae
		Prymnesiovirus	*Chryosochromomulina brevifilium virus PW1*	Algae
		Phaeovirus	*Extocarpus siliculosus virus 1*	Algae
		Coccolithovirus	*Emiliania huxleyi virus 86*	Algae
		Raphidovirus	*Heterosigms akashiwo virus 01*	Algae
Plasmaviridae		*Plasmavirus*	*Acholeplasma phage L2*	Mycoplasma
Polydnaviridae		*Ichnovirus*	*Campoletis sonorensis ichnovirus*	Invertebrates
		Bracovirus	*Cotesia melanoscela brachovirus*	Invertebrates
Poxviridae:	*Chordopoxvirinae*	*Orthopoxvirus*	*Vaccinia virus*	Vertebrates
		Parapoxvirus	*Orf virus*	Vertebrates
		Avipoxvirus	*Fowlpox virus*	Vertebrates
		Capripoxvirus	*Sheeppox virus*	Vertebrates
		Leporipoxvirus	*Myxoma virus*	Vertebrates
		Suipoxvirus	*Swinepox virus*	Vertebrates
		Molluscipoxvirus	*Molluscum contagiosum virus*	Vertebrates
		Yatapoxvirus	*Yaba monkey tumor virus*	Vertebrates

(continued)

Table 4.10 (continued)

Order *Caudovirales* – tailed bacteriophages				
Family				
Subfamily		Genus	Type species	Hosts
	Entomopoxvirinae	*Entomopoxvirus A*	*Melolontha melolontha entomopoxvirus*	Invertebrates
		Entomopoxvirus B	*Amsacta moorei entomopoxvirus*	Invertebrates
		Entomopoxvirus C	*Chironomus luridus entomopoxvirus*	Invertebrates
Rhizidovirus			*Rhizidomyces virus*	Fungi
Rudiviridae		*Rudivirus*	*Sulfolobus virus SIRV1*	Archaea
Tectiviridae		*Tectivirus*	*Enterobacteria phage PRD1*	Bacteria

Table 4.11 Group II: The ssDNA viruses (Reproduced from Van Regenmortel et al. 2005; http://www.microbiologybytes.com/virology/VirusGroups.html#VI; Anonymous 2005. With permission)

Group II: ssDNA viruses				
Family				
Subfamily		Genus	Type species	Hosts
Anellovirus			*Torque teno virus*	Vertebrates
Circoviridae		*Circovirus*	*Porcine circovirus*	Vertebrates
		Gyrovirus	*Chicken anemia virus*	Vertebrates
Geminiviridae		*Mastrevirus*	*Maize streak virus*	Plants
		Curtovirus	*Beet curly top virus*	Plants
		Topocuvirus	*Tomato pseudo-curly top virus*	Plants
		Begomovirus	*Bean golden mosaic virus*	Plants
Inoviridae		*Inovirus*	*Enterobacteria phage M13*	Bacteria
		Plectrovirus	*Acholeplasma phage MV-L51*	Bacteria
Microviridae		*Microvirus*	*Enterobacteria ØX174*	Bacteria
		Spiromicrovirus	*Spiroplasma phage 4*	Spiroplasma
		Bdellomicrovirus	*Bdellovibrio phage MAC1*	Bacteria
		Chlamydiamicrovirus	*Chlamydia phage 1*	Bacteria
Nanoviridae		*Nanovirus*	*Subterranean clover stunt virus*	Plants
		Babuvirus	*Banana bunchy top virus*	Plants
Parvoviridae:	*Parvovirinae*	*Parvovirus*	*Mice minute virus*	Vertebrates
		Erythrovirus	*B19 virus*	Vertebrates
		Dependovirus	*Adeno-associated virus 2*	Vertebrates
		Amdovirus	*Aleutian mink disease virus*	Vertebrates
		Bocavirus	*Bovine parvovirus*	Vertebrates
	Densovirinae	*Densovirus*	*Junonia coenia densovirus*	Invertebrates
		Iteravirus	*Bombyx mori densovirus*	Invertebrates
		Brevidensovirus	*Aedes aegypti densovirus*	Invertebrates
		Pefudensovirus	*Periplanta fuliginosa densovirus*	Invertebrates
		Circovirus	*Porcine circovirus*	Vertebrates

4.1.7.3 Bacteriophages in the Aquatic Environment

Until recently, it was thought that aquatic environments, marine and freshwater, were devoid of viruses. New techniques now show them to be abundant in the aquatic environment, where they contribute to nutrient cycle by lysing microorganisms. All microorganisms, bacteria (bacteriophages), fungi (mycophages), algae (phycophages), and protozoa are attacked by viruses (phages, phago = eat, Greek) which attack (eat) them.

Table 4.12 Group III: dsRNA viruses (Reproduced from Van Regenmortel et al. 2005; http://www.microbiologybytes.com/virology/VirusGroups.html#VI; Anonymous 2005. With permission)

Family			
Subfamily	Genus	Type species	Hosts
Birnaviridae	*Aquabirnavirus*	*Infectious pancreatic necrosis virus*	Vertebrates
	Avibirnavirus	*Infectious bursal disease virus*	Vertebrates
	Entomobirnavirus	*Drosophila X virus*	Invertebrates
Chrysoviridae	*Chrysovirus*	*Penicillium chrysogenum virus*	Fungi
Cystoviridae	*Cystovirus*	*Pseudomonas phage Ø6*	Bacteria
Endornavirus		*Vicia faba endornavirus*	Plants
Hypoviridae	*Hypovirus*	*Cryphonectria hypovirus 1-EP713*	Fungi
Partitiviridae	*Partitivirus*	*Atkinsonella hypoxylon virus*	Fungi
	Alphacryptovirus	*White clover cryptic virus 1*	Plants
	Betacryptovirus	*White clover cryptic virus 2*	Plants
Reoviridae	*Orthoreovirus*	*Mammalian orthoreovirus*	Vertebrates
	Orbivirus	*Bluetongue virus*	Vertebrates
	Rotavirus	*Rotavirus A*	Vertebrates
	Coltivirus	*Colorado tick fever virus*	Vertebrates
	Aquareovirus	*Golden shiner virus*	Vertebrates
	Seadornavirus	*Banna virus*	Vertebrates
	Cypovirus	*Cypovirus 1*	Invertebrates
	Idnoreovirus	*Idnoreovirus 1*	Invertebrates
	Fijivirus	*Fiji disease virus*	Plants
	Phytoreovirus	*Wound tumor virus*	Plants
	Oryzavirus	*Rice ragged stunt virus*	Plants
	Mycoreovirus	*Mycoreovirus 1*	Fungi
Totiviridae	*Totivirus*	*Saccharomyces cerevisiae virus L-A*	Fungi
	Giardiavirus	*Giardia lamblia virus*	Protozoa
	Leishmaniavirus	*Leishmania RNA virus 1-1*	Protozoa

Table 4.13 Group IV: (+)sense RNA viruses (Reproduced from Van Regenmortel et al. 2005; http://www.microbiologybytes.com/virology/VirusGroups.html#VI; Anonymous 2005. With permission)

Order *Nidovirales* – "Nested" viruses			
Family			
Subfamily	Genus	Type species	Hosts
Arteriviridae	*Arterivirus*	*Equine arteritis virus*	Vertebrates
Coronaviridae	*Coronavirus*	*Infectious bronchitis virus*	Vertebrates
	Torovirus	*Equine torovirus*	Vertebrates
Roniviridae	*Okavirus*	*Gill-associated virus*	Vertebrates
Astroviridae	*Avastrovirus*	*Turkey astrovirus*	Vertebrates
	Mamastrovirus	*Human astrovirus*	Vertebrates
Barnaviridae	*Barnavirus*	*Mushroom bacilliform virus*	Fungi
Benyvirus		*Beet necrotic yellow vein virus*	Plants
Bromoviridae	*Alfamovirus*	*Alfalfa mosaic virus*	Plants
	Bromovirus	*Brome mosaic virus*	Plants
	Cucumovirus	*Cucumber mosaic virus*	Plants
	Ilarvirus	*Tobacco streak virus*	Plants
	Oleavirus	*Olive latent virus 2*	Plants

(continued)

Table 4.13 (continued)

Order *Nidovirales* – "Nested" viruses			
Family			
Subfamily	Genus	Type species	Hosts
Caliciviridae	*Lagovirus*	*Rabbit haemorrhagic disease virus*	Vertebrates
	Norovirus	*Norwalk virus*	Vertebrates
	Sapovirus	*Sapporo virus*	Vertebrates
	Vesivirus	*Swine vesicular exanthema virus*	Vertebrates
Cheravirus		*Cherry rasp leaf virus*	Plants
Closteroviridae	*Ampelovirus*	*Grapevine leafroll-associated virus 3*	Plants
	Closterovirus	*Beet yellows virus*	Plants
Comoviridae	*Comovirus*	*Cowpea mosaic virus*	Plants
	Fabavirus	*Broad bean wilt virus 1*	Plants
	Nepovirus	*Tobacco ringspot virus*	Plants
Dicistroviridae	*Cripavirus*	*Cricket paralysis virus*	Invertebrates
Flaviviridae	*Flavivirus*	*Yellow fever virus*	Vertebrates
	Pestivirus	*Bovine diarrhea virus 1*	Vertebrates
	Hepacivirus	*Hepatitis C virus*	Vertebrates
Flexiviridae	*Potexvirus*	*Potato virus X*	Plants
	Mandarivirus	*Indian citrus ringspot virus*	Plants
	Allexivirus	*Shallot virus X*	Plants
	Carlavirus	*Carnation latent virus*	Plants
	Foveavirus	*Apple stem pitting virus*	Plants
	Capillovirus	*Apple stem grooving virus*	Plants
	Vitivirus	*Grapevine virus A*	Plants
	Trichovirus	*Apple chlorotic leaf spot virus*	Plants
Furovirus		*Soil-borne wheat mosaic virus*	Plants
Hepevirus		*Hepatitis E virus*	Vertebrates
Hordeivirus		*Barley stripe mosaic virus*	Plants
Idaeovirus		*Rasberry bushy dwarf virus*	Plants
Iflavirus		*Infectious flacherie virus*	Invertebrates
Leviviridae	*Levivirus*	*Enterobacteria phage MS2*	Bacteria
	Allolevivirus	*Enterobacteria phage Qβ*	Bacteria
Luteoviridae	*Luteovirus*	*Cereal yellow dwarf virus-PAV*	Plants
	Polerovirus	*Potato leafroll virus*	Plants
	Enamovirus	*Pea enation mosaic virus-1*	Plants
Machlomovirus		*Maize chlorotic mottle virus*	Plants
Marnaviridae	*Marnavirus*	*Heterosigma akashiwo RNA virus*	Fungi
Narnaviridae	*Narnavirus*	*Saccharomyces cerevisiae narnavirus 20S*	Fungi
	Mitovirus	*Cryphonectria parasitica mitovirus 1-NB631*	Fungi
Nodaviridae	*Alphanodoavirus*	*Nodamura virus*	Invertebrates
	Betanodovirus	*Striped jack nervous necrosis virus*	Vertebrates
Pecluvirus		*Peanut clump virus*	Plants
Ourmiavirus		*Ourmia melon virus*	Plants
Picornaviridae	*Enterovirus*	*Poliovirus*	Vertebrates
	Rhinovirus	*Human rhinovirus A*	Vertebrates
	Hepatovirus	*Hepatitis A virus*	Vertebrates
	Cardiovirus	*Encephalomyocarditis virus*	Vertebrates
	Aphthovirus	*Foot-and-mouth disease virus O*	Vertebrates
	Parechovirus	*Human parechovirus*	Vertebrates
	Erbovirus	*Equine rhinitis B virus*	Vertebrates
	Kobuvirus	*Aichi virus*	Vertebrates
	Teschovirus	*Porcine teschovirus*	Vertebrates

(continued)

Table 4.13 (continued)

Order *Nidovirales* – "Nested" viruses			
Family			
Subfamily	Genus	Type species	Hosts
Pomovirus		*Potato mop-top virus*	Plants
Potyviridae	*Potyvirus*	*Potato virus Y*	Plants
	Ipomovirus	*Sweet potato mild mottle virus*	Plants
	Macluravirus	*Maclura mosaic virus*	Plants
	Rymovirus	*Ryegrass mosaic virus*	Plants
	Tritimovirus	*Wheat streak mosaic virus*	Plants
	Bymovirus	*Barley yellow mosaic virus*	Plants
Sadwavirus		*Satsuma dwarf virus*	Plants
Sequiviridae	*Sequivirus*	*Parsnip yellow fleck virus*	Plants
	Waikavirus	*Rice tungro spherical virus*	Plants
Sobemovirus		*Southern bean mosaic virus*	Plants
Tetraviridae	*Betatetravirus*	*Nudaurelia capensis β virus*	Invertebrates
	Omegatetravirus	*Nudaurelia capensis ω virus*	Invertebrates
Tobamovirus		*Tobacco mosaic virus*	Plants
Tobravirus		*Tobacco rattle virus*	Plants
Tombusviridae	*Tombusvirus*	*Tomato bushy stunt virus*	Plants
	Avenavirus	*Oat chlorotic stunt virus*	Plants
	Aureusvirus	*Pothos latent virus*	Plants
	Carmovirus	*Carnation mottle virus*	Plants
	Dainthovirus	*Carnation ringspot virus*	Plants
	Machlomovirus	*Maize chlorotic mottle virus*	Plants
	Necrovirus	*Tobacco necrosis virus*	Plants
	Panicovirus	*Panicum mosaic virus*	Plants
Togaviridae	*Alphavirus*	*Sindbis virus*	Vertebrates
	Rubivirus	*Rubella virus*	Vertebrates
Tymoviridae	*Maculavirus*	*Grapevine fleck virus*	Plants
	Marafivirus	*Maize rayado fino virus*	Plants
	Tymovirus	*Turnip yellow mosaic virus*	Plants
Umbravirus		*Carrot mottle virus*	Plants

Table 4.14 Group V: (–) sense RNA viruses (Reproduced from Van Regenmortel et al. 2005; http://www.microbiologybytes.com/virology/VirusGroups.html#VI; Anonymous 2005. With permission)

Order Mononegavirales				
Family				
Subfamily		Genus	Type species	Hosts
Bornaviridae		*Bornavirus*	*Borna disease virus*	Vertebrates
Filoviridae		*Marburgvirus*	*Lake Victoria marburgvirus*	Vertebrates
		Ebolavirus	*Zaire ebolavirus*	Vertebrates
Paramyxoviridae	*Paramyxovirinae*	*Avulavirus*	*Newcastle disease virus*	Vertebrates
		Henipavirus	*Hendra virus*	Vertebrates
		Morbillivirus	*Measles virus*	Vertebrates
		Respirovirus	*Sendai virus*	Vertebrates
		Rubulavirus	*Mumps virus*	Vertebrates
	Pneumovirinae	*Pneumovirus*	*Human respiratory syncytial virus*	Vertebrates
		Metapneumovirus	*Avian pneumovirus*	Vertebrates

(continued)

Table 4.14 (continued)

Order Mononegavirales			
Family			
Subfamily	Genus	Type species	Hosts
Rhabdoviridae	*Vesiculovirus*	*Vesicular stomatitis Indiana virus*	Vertebrates, invertebrates
	Lyssavirus	*Rabies virus*	Vertebrates
	Ephemerovirus	*Bovine ephemeral fever virus*	Vertebrates, invertebrates
	Novirhabdovirus	*Infectious hematopoetic necrosis virus*	Vertebrates
	Cytorhabdovirus	*Lettuce necrotic yellows virus*	Plants, invertebrates
	Nucleorhabdovirus	*Potato yellow dwarf virus*	Plants, invertebrates
Arenaviridae	*Arenavirus*	*Lymphocytic choriomeningitis virus*	Vertebrates
Bunyaviridae	*Orthobunyavirus*	*Bunyamwera virus*	Vertebrates
	Hantavirus	*Hantaan virus*	Vertebrates
	Nairovirus	*Nairobi sheep disease virus*	Vertebrates
	Phlebovirus	*Sandfly fever Sicilian virus*	Vertebrates
	Tospovirus	*Tomato spotted wilt virus*	Plants
Deltavirus		*Hepatitis delta virus*	Vertebrates
Ophiovirus		*Citrus psorosis virus*	Plants
Orthomyxoviridae	*Influenza A virus*	*Influenza A virus*	Vertebrates
	Influenza B virus	*Influenza B virus*	Vertebrates
	Influenza C virus	*Influenza C virus*	Vertebrates
	Isavirus	*Infectious salmon anemia virus*	Vertebrates
	Thogotovirus	*Thogoto virus*	Vertebrates
Tenuivirus		*Rice stripe virus*	Plants
Varicosaivirus		*Lettuce big-vein associated virus*	Plants

Note: Families are in uppercase and genera in bold starting with capital letters. The Order is represented in this case and is in larger display than the family
RNA viruses are also designated according to the sense or polarity of their RNA into negative-sense and positive-sense, or **ambisense.** Positive-sense viral RNA is similar to mRNA and thus can be immediately translated by the host cell. Negative-sense viral RNA is complementary to mRNA and thus must be converted to positive-sense RNA by an RNA polymerase before translation. Ambisense RNA viruses resemble negative-sense RNA viruses, except they also translate genes from the positive strand. They differ from those of other negative-sense RNA viruses in that some proteins are coded in viral-complementary RNA sequences and others are coded in the viral RNA sequence

Bacteriophages are the most abundant among the phages and they have been more widely studied. Bacteriophages were first formally described by the French Canadian Felix d' Herelle in 1915, but the initial observations were made during 1896, followed by observations made by the British bacteriologist Frederick Twort in 1913 (see Fig. 4.26). On account of their importance in aquatic systems, the life cycle of bacterial viruses, the methods of isolating and enumerating them from water, their grouping, and their host range will be discussed below.

Life History of Bacteriophages

When bacteriophages enter susceptible bacteria, they take over the genetic apparatus of their hosts and force the hosts to produce more viruses of their type. When the virions (virus particles) mature, they produce enzymes which lyse the host cell wall releasing the virus particle to start life afresh. When they lyse the host they are in the lytic phase.

Sometimes they enter into a phase, the lysogenic phase, in which the phages remain in the cell and replicate with it. This phase is the lysogenic phase (see Fig. 4.27).

Table 4.15 Group VI: reverse transcribing Diploid single-stranded RNA viruses (Reproduced from Van Regenmortel et al. 2005; http://www.microbiologybytes.com/virology/VirusGroups.html#VI; Anonymous 2005. With permission)

Family			
Subfamily	Genus	Type species	Hosts
Retroviridae	*Alpharetrovirus*	*Avian leukosis virus*	Vertebrates
	Betaretrovirus	*Mouse mammary tumor virus*	Vertebrates
	Gammaretrovirus	*Murine leukemia virus*	Vertebrates
	Deltaretrovirus	*Bovine leukemia virus*	Vertebrates
	Epsilonretrovirus	*Walley dermal sarcoma virus*	Vertebrates
	Lentivirus	*Human immunodeficiency virus 1*	Vertebrates
	Spumavirus	*Human spumavirus*	Vertebrates
Metaviridae	*Metavirus*	*Saccharomyces cerevisiae Ty3 virus*	Fungi
	Errantivirus	*Drosophila melanogaster gypsy virus*	Invertebrates
Pseudoviridae	*Pseudovirus*	*Saccharomyces cerevisiae Ty1 virus*	Invertebrates
	Hemivirus	*Drosophila melanogaster copia virus*	Invertebrates

Table 4.16 Group VII: DNA reverse transcribing viruses (Reproduced from Van Regenmortel et al. 2005; http://www.microbiologybytes.com/virology/VirusGroups.html#VI; Anonymous 2005. With permission)

Family			
Subfamily	Genus	Type species	Hosts
Hepadnaviridae	*Orthohepadnavirus*	*Hepatitis B virus*	Vertebrates
	Avihepadnavirus	*Duck hepatitis B virus*	Vertebrates
Caulimoviridae	*Caulimovirus*	*Cauliflower mosaic virus*	Plants
	Badnavirus	*Commelina yellow mottle virus*	Plants
	Cavemovirus	*Cassava vein mosaic virus*	Plants
	Petuvirus	*Petunia vein clearing virus*	Plants
	Soymovirus	*Soybean chlorotic mottle virus*	Plants
	Tungrovirus	*Rice tungro bacilliform virus*	Plants

Table 4.17 The structures of DNAviruses (From http://en.wikipedia.org/wiki/Virus_classification; Anonymous 2010a)

S. No.	Virus family	Virus genus	Virion – naked/enveloped	Capsid symmetry	Type of nucleic acid
1.	Adenoviridae	Adenovirus	Naked	Icosahedral	ds
2.	Papovaviridae	Papillomavirus	Naked	Icosahedral	ds circular
3.	Parvoviridae	B 19 virus	Naked	Icosahedral	ss
4.	Herpesviridae	Herpes Simplex Virus, Varicella zoster virus, Cytomegalovirus, Epstein Barr virus	Enveloped	Icosahedral	ds
5.	Poxviridae	Small pox virus, Vaccinia virus	Complex coats	Complex	ds
6.	Hepadnaviridae	Hepatitis B virus	Enveloped	Icosahedral	ds circular
7.	Polyomaviridae	Polyoma virus (progressive multifocal leucoencephalopathy)	?	?	ds

Table 4.18 The structure of RNA viruses (From http://en.wikipedia.org/wiki/Virus_classification; Anonymous 2010a)

S. No.	Virus family	Virus genus	Virion – naked/enveloped	Capsid symmetry	Type of nucleic acid
1.	Reoviridae	Reovirus, Rotavirus	Naked	Icosahedral	ds
2.	Picornaviridae	Poliovirus, Rhinovirus, Hepatitis A virus	Naked	Icosahedral	ss
3.	Caliciviridae	Norwalk virus, Hepatitis E virus	Naked	Icosahedral	ss
4.	Togaviridae	Rubella virus	Enveloped	Icosahedral	ss
5.	Arenaviridae	Lymphocytic choriomeningitis virus	Enveloped	Complex	ss
6.	Retroviridae	HIV-1, HIV-2, HTLV-I	Enveloped	Complex	ss
7.	Flaviviridae	Dengue virus, hepatitis C virus, yellow fever virus	Enveloped	Complex	ss
8.	Orthomyxoviridae	Influenza virus	Enveloped	Helical	ss
9.	Paramyxoviridae	Measles virus, mumps virus, respiratory syncytial virus	Enveloped	Helical	ss
10.	Bunyaviridae	California encephalitis virus, Hantavirus	Enveloped	Helical	ss
11.	Rhabdoviridae	Rabies virus	Enveloped	Helical	ss
12.	Filoviridae	Ebola virus, Marburg virus	Enveloped	Helical	ss
13.	Coronaviridae	Corona virus	Enveloped	Complex	ss
14.	Astroviridae	Astro virus	Naked	Icosahedral	ss
15.	Bornaviridae	Borna disease virus	Enveloped	Helical	ss

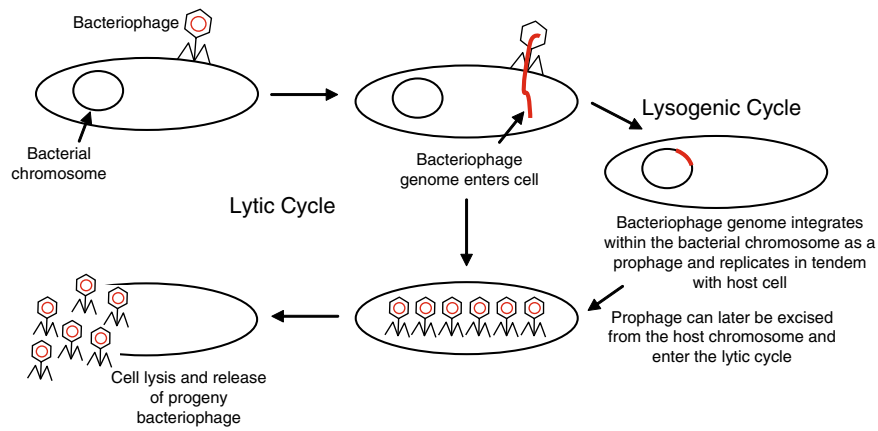


Fig. 4.27 (The lytic and lysogenic cycles of bacteriophage replication (from Zourob & Ripp, 2010, with kind permission from Springer Science+Business Media: Zourob, M. & Ripp, S. (2010). Bacteriophage-Based Biosensors. In M. Zourob (Ed.) Recognition Receptors in Biosensors (Fig. 11.3, p. 419). New York: Springer)

Methods for the Study of Bacteriophages

Because of their small size, bacteriophages are not usually studied directly as is the case with microorganisms. An electron microscope is necessary to study viruses, but not only are electron microscopes expensive, but they require skilled operators to handle them. Therefore, viruses are studied indirectly through their effects. The indirect methods used especially for animal and plant viruses, include the changes (known as cytopathic effects, CPE) they bring about in the cells in the cell culture in which they are grown: lysis, altered shape, detachment from substrate, membrane fusion, altered membrane permeability.

Other methods for studying viruses, particularly of animals and plants, are serological methods based on the interaction between virus and antibody produced specifically against it, detection of viral nucleic acid,

including the use of polymerase chain reaction (PCR) for the detection of DNA or RNA. These methods are used mostly for studying animal and plant viruses. For bacteriophages, the chief method of detection is the cytopathic effect (CPE).

Isolation and Enumeration of Bacteriophages

Bacteriophages are sometimes very abundant in water and because of the specificity of the bacteria they attack, it has been suggested that they can be used as indicators of fecal pollution of water.

Bacteriophages may be isolated and/or enumerated from water in the following ways (McLaughlin et al. 2006):

(a) *Enrichment*
This method is used if the aim is to isolate phages attacking a particular bacterium from the water sample. A pure culture of the bacterium whose phages are to be isolated is introduced into sample of the water to be assessed for phage load, say about 1 ml of a log phase culture of the bacterium is added to about 10–15 ml of the water and incubated under conditions which will encourage the growth of the bacterium, including shaking if necessary. At the end of 18–24 h growth, a quantity of chloroform is added to kill the bacteria, and the broth is filtered in a 0.45 μm filter to remove the debris. The filtrate is then serially diluted and about 0.5–1 ml of the dilutions mixed with molten agar at 45°C and poured in to plates and incubated. It is assumed that each zone of clearing (plaque) on the bacterial lawn indicates one bacteriophage, or more correctly, one plaque forming unit (PFU), since as the case with bacteria, a clearing could be formed by a clump of bacteriophages. To ensure the avoidance of clumps, the counting or selection should done with as high a dilution as possible; the filtrate can also be shaken to breakup clumps before introduction into the agar.

Some workers prefer to use the soft agar method. In this method, a small volume of a dilution of phage suspension and a small quantity of host cells grown to high cell density, sufficient to give 10^7–10^8 CFU/ml (colony forming units/ml), are mixed in about 2.5 ml of molten, "soft" agar at 46°C. The resulting suspension is then poured on to an appropriate basal agar medium. This poured mixture cools and forms a thin "top layer" which hardens and immobilizes the bacteria.

(b) *Direct plating out*
If the aim is to assess the diversity of phages present in the water body, several dilutions of the water are made. At each dilution, pour plates are made as described above, each plate with one of the variety of bacteria whose phages are being sought in the water.

(c) *Direct counting in a flow cytometer*
In this method, the water may be centrifuged to concentrate the virus. The phages are stained with highly fluorescent nucleic acid specific dyes such as SYBR Green I, SYBR Green II, OliGreen, or PicoGreen. Flow cytometry allows extremely rapid enumeration of single cells, primarily by optical means. Cells scatter light when passing through the laser beam and emit fluorescent light when excited by the laser. Flow cytometry has become an invaluable tool for both qualitative and quantitative analyses owing to its rapidity. It has been used as a rapid method for detecting viruses from different families. Flow cytometry appears faster and more accurate than any other method currently used for the direct detection and quantification of virus particles (Brussaard et al. 2000).

Although epifluorescence microscopy is commonly used for the enumeration of bacteria and other microorganisms in natural water samples including viruses, because of its simplicity and ready availability of the microscopes, distinguishing viruses based on differences in fluorescence intensity is difficult with the epifluorscence microscope; small bacteria may for example be counted for large viruses.

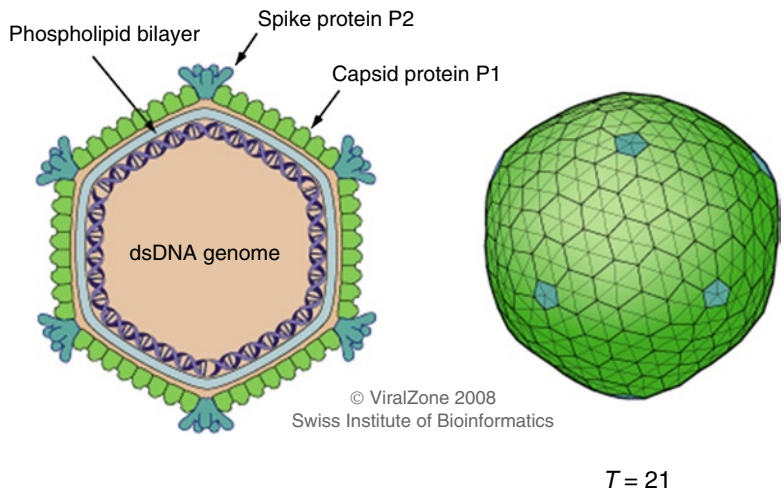
Bacteriophages and Their Bacterial Hosts

Figure 4.28 gives the names of the bacteriophages, the description of their virions and the hosts which they attack. It will be seen that in many cases, the same bacteriophages may sometimes attack numerous hosts, while in some cases, a bacteriophage is restricted to one host.

4.1.8 Small Multicellular Macroorganisms in Aquatic Systems

Two groups of small multicellular macroorganisms occur in water, namely, crustaceans (including rotifers) and nematodes.

1 CORTICOVIRIDAE

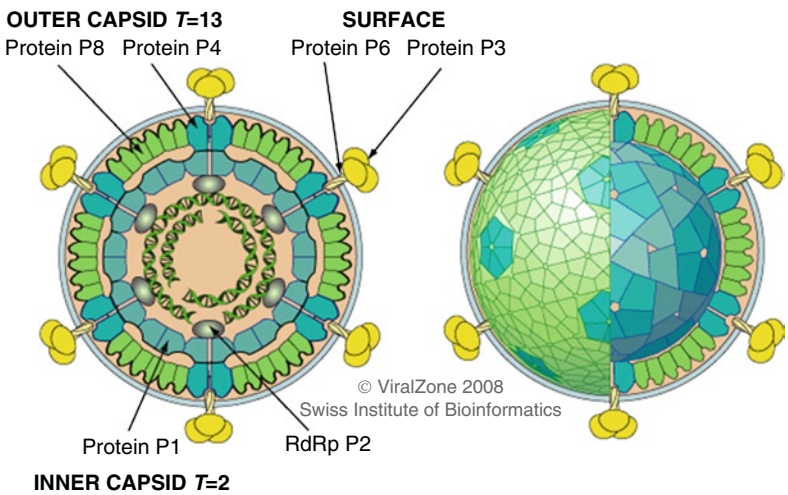


Structure
Phages consist of a capsid and an internal lipid membrane. Virus capsid is **not enveloped**. Internal lipid membrane located between outer and inner protein shell. Capsid/nucleocapsid is round and exhibits icosahedral symmetry (T=12), or 13). The capsid is isometric and has a diameter of 60 nm (or more). The capsid shells of virions are composed of three layers. Capsids appear hexagonal in outline. The capsid surface structure reveals a regular pattern with distinctive features. The capsomer arrangement is clearly visible. Surface projections are distinct, brush-like spikes protruding from the 12 vertices. Capsids all have the same appearance.

The genome is not segmented, constitutes 13% of the virus's weight and contains a single molecule of circular, supercoiled, double-stranded DNA of 9500-12000 nucleotides in length. The genome has a g + c content of 43%.

Host
Their hosts are members of the Phylum *Proteobacteria*.

2 CYSTOVIRIDAE



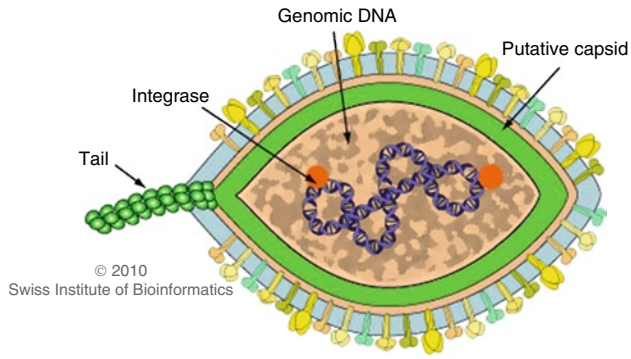
Structure
Enveloped, spherical virion of 85 nm in diameter. The virion has a double capsid structure: Outer capsid a has an icosahedral T=13 symmetry, inner capsid an icosahedral symmetry T=2.

All cystoviruses are distinguished by their three strands (analogous to chromosomes) of double stranded (ds) RNA, totalling ~14 kb in length and their protein and lipid outer layer. No other bacteriophage have any lipid in their outer coat, though the *Tectiviridae* and the *Corticoviridae* have lipids within their capsids.

Members of the *Cystoviridae* appear to be most closely related to the *Reoviridae* but also share homology with the *Totiviridae*. Cystoviruses are the only bacteriophage that are more closely related to viruses of eukaryotes than to other phage.

Host
Most identified cystoviruses infect *Pseudomonas* species, but this is likely biased due to the method of screening and enrichment. The type species is *Pseudomonas phage Φ6*, but there are many other members of this family. Φ7, Φ8, Φ9, Φ10, Φ11, Φ12 and Φ13 have been identified and named, but other cystoviruses have also been isolated.

3 FUSSELLOVIRIDAE



Structure

Enveloped, lemon-shaped, with short tail fibers attached to one pole. The virion is about 60 nm in diameter and 100 nm in length.

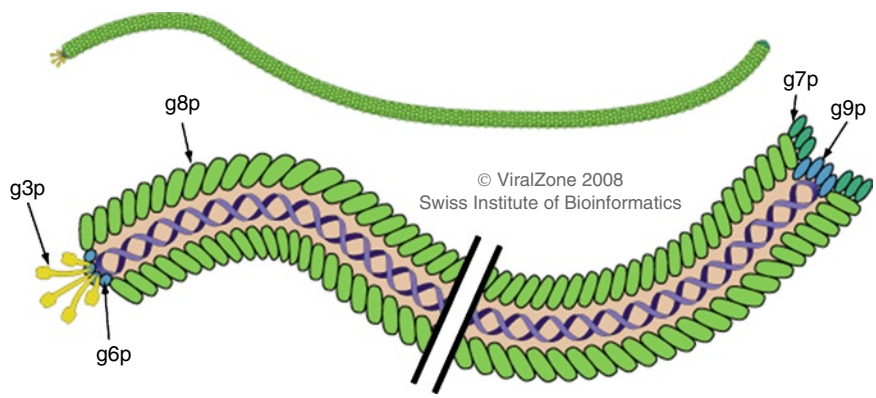
The genome of a fuselloviridae is non-segmented and contains a single molecule of circular, double-stranded DNA. The DNA is positively supercoiled. The complete genome is 15500 nucleotides in length; encodes for 31 to 37 genes.

Fuselloviridae virions consist of an envelope and a nucleocapsid. The capsid is enveloped. Virions are spindle-shaped, flexible, and have protrusions that extend through the envelope. One pole has short tail-like fibers attached to it. The virions are 100 nm in length and 60 nm in diameter.

Host

Fuselloviridae infect the Archae *Sulfolobus* which inhabits high-temperature (>70°C), acidic (pH of <4.0) environments. Members of this family have been found in acidic hotsprings in Japan and Iceland. The Fuselloviridae family currently consists of only one virus, *Sulfolobus* spindle-shaped virus 1 (SSV1), and three tentative members (SSV2, SSV3, and the staaelite virus pSSVx, which stands for plasmid SSV x). SSV1, the type virus for the family, was the first high-temperature virus to be characterized.

4 INOVIRIDAE



Structure

Non-enveloped, Rod of filaments ut 7nm in diameter and from 700 to 2000nm long.

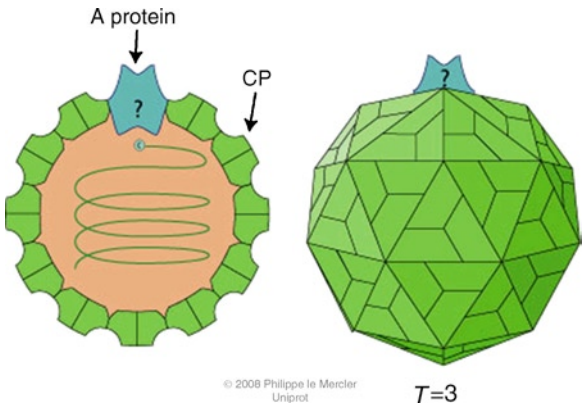
Circular, single-stranded DNAof 4.5 to 8kb encoding for 4 to 10 proteins. Replication occurs via dsDNA intermediate and rolling circle.

Host

Members of the family infect their natural hosts without causing lysis, and the infected cells continue to divide and produce virus indefinitely. The hosts are plant and animal pathogens. In several systems the phage enter into lysogenic phases.

lnouirus hosts are all gram-negative bacteria (i.e., *Escherichia coli, Salmonella, Pseudomonas, Vibrio, Xanthomonas,* etc.). Host ranges are determined primarily by host cell receptors, which are usually conjugative pili. Some pili are encoded chromosomally and some are encoded on plasmids of different incompatibility groups. Transmission of the plasmids to new bacterial species usually transfers phage sensitivity. Additional host range determinants include restriction-modification systems, host periplasmic proteins involved in viral ssDNA translocation into the cytoplasm, and host protein(s) involved in membrane assembly. Transfections of non-natural hosts with naked ssDNA or dsDNA are sometimes possible. When *Vibrio cholera* phage lysogens colonize the human intestine, states of elevated cholera toxin expression and release, and of progeny filamentous choleraphage extrusion, are induced. Thus *lnaoirus* lysogeny is a critical virulence factor in cholera pathogenesis.

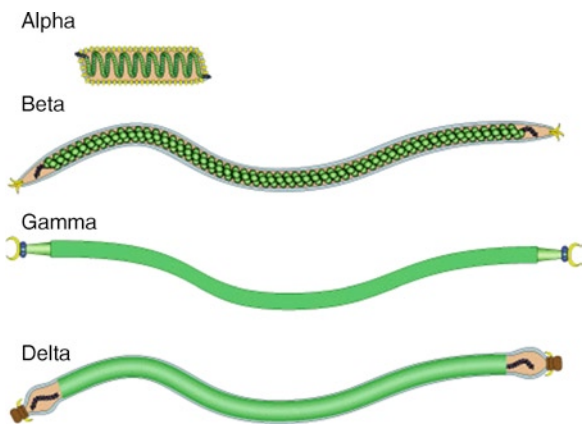
5 LEVIVIRIDAE



Structure
Non-enveloped, spherical virion about 26nm in diameter with icosahedral symmetry (T=3) composed of 180 CP proteins and a single A protein. Linear, ssRNA(+) genome about 4 kb in size. The 5' end is capped. Encodes for 4 proteins.

Host
It attacks Enterobacteriacea, Acinetobacter, Caulobacter and Pseudomonas.

6 LIPOTHRIXVIRIDAE



Structure
Enveloped, rod-shaped. The capsid is about 24-38 nm in diameter and 410-1950 nm in length.

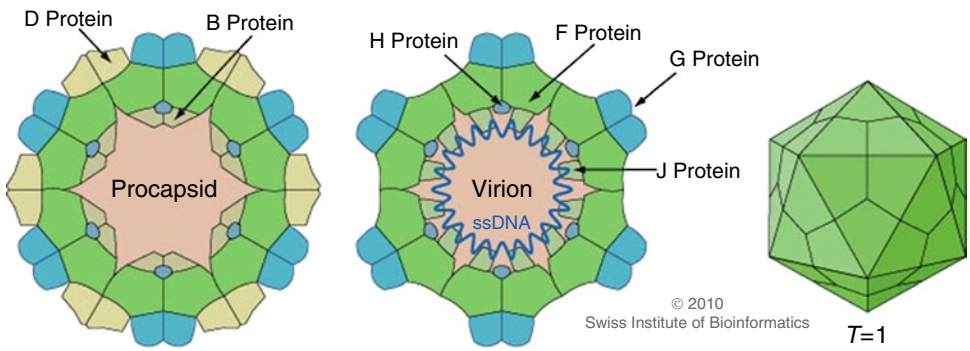
Linear dsDNA genome of 15.9 to 56 kb. Extremities of the DNAare modified in an unknown manner.

The Lipothrixviridae family consists of a family of viruses that infect archaea. They share characteristics from the Rudiviridae family and both have are filamentous viruses with linear dsDNA genomes that infect thermophilic archaea in the kingdom Crenarchaeota. Lipothrixviridae are enveloped.

Host
Lipothrixviridae is a family crenarchaeal viruses. It is by far the most diverse family of crenarchaeal viruses, with six isolates divided into three genera: *Alphalipothrixvirus*, *Betalipothrixvirus*, and *Gammalipothrixvirus*. *Alphalipothrixvirus* contains TTV1, TTV2, and TTV3, isolated from acidic hot springs Iceland. *Betalipothrixvirus* contains SIFV, also isolated in Iceland. Finally, *Gammalipothrixvirus* is represented by AFV1, isolated from Yellowstone National Park.

Also Acidianus, Sulfolobus, Thermoproteus.

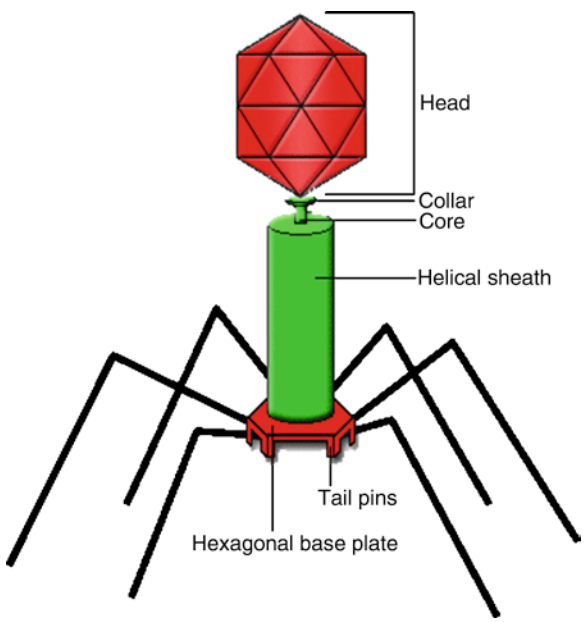
7 MICROVIRIDAE



Structure
Non-enveloped, round, icosahedral symmetry (T=1), about 30 nm in diameter. The capsid consists of 12 pentagonal trumpet-shaped pentomers. The virion is composed of 60 copies each of the F, G, and J proteins, and 12 copies of the H protein. There are 12 spikes which are each composed of 5 G and one H proteins.

Host
Attacks Bdellovibrio, Chlamydia, Enterobacteria, Spiroplasma, Enterobacteria Clamydiamicrovirus and Bdellomicrovirus: intracellular parasitic **bacteria**. Spiromicrovirus: Spiroplasma.

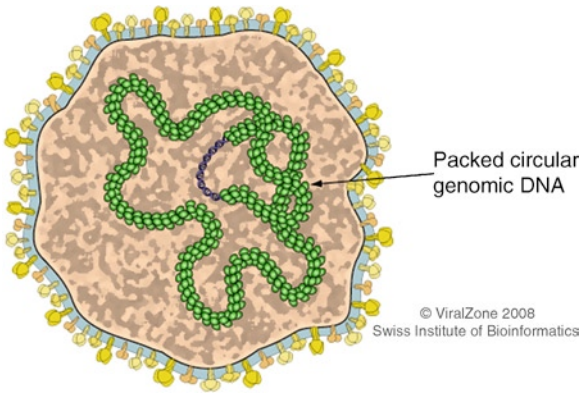
8 Myoviridae



Structure
Myoviruses are not enveloped and consist of a head and a tail separated by a neck. The head has icosahedral symmetry, while the tail is tubular and has helical symmetry. The capsid that constitutes the head is made up of 152 capsomers. The head has a diameter of 50-110nm; the tail is 16-20nm in diameter. The tail consists of a central tube, a contractile sheath, a collar, a base plate, six tail pins and six long fibers. Tail structure is similar to tectiviridae, but differs in the fact that a myovirus' tail is permanent. Contractions of the tail require ATP. When the sheath is contracted, it measures 10-15 nm in lengthicosahedral capsid, circular ssDNA.

Host
Myoviruses, being bacteriophages, infect bacteria. The most commonly infected bacteria is *Escherichia coli*. Myoviruses are virulent phages, meaning they do not integrate their genetic material with their host cell's, and they usually kill their host cell. Others are Bdellovibrio, Chlamydia, Enterobacteria, Spiroplasma.

9 PLASMAVIRIDAE



Structure
Pleomorphic, envelope, lipids, no capsid, circular supercoiled dsDNA. Enveloped, spherical to pleomorphic, no head-tail structure. The capsid is about 80 nm in diameter.

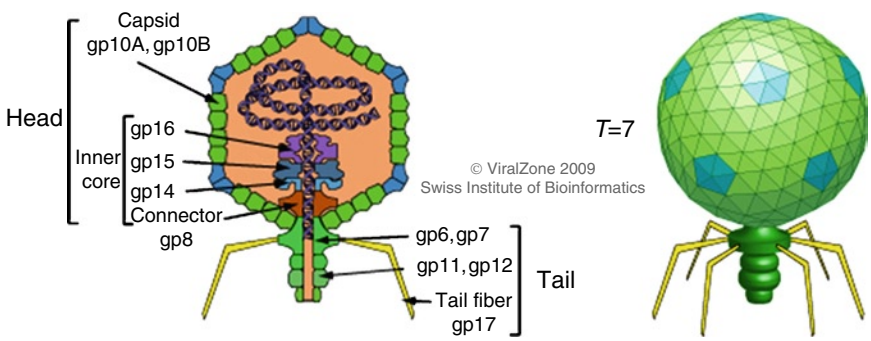
The Plasmaviridae is a family of bacteriophages, viruses that infects bacteria. Virions have an envelope, a nucleoprotein complex, and a capsid. They are 50-125 nm in diameter with a baggy or loose membrane.

The genome is condensed, non segmented and consists of a single molecule of circular, supercoiled double-stranded DNA, 12000 base pairs in length. The genome has a rather high G-C content of around 32 percent.

A productive infectious cycle begins before a lysogenic cycle establishes the virus in the infected bacteria. After initial infection of the viral genome the virus may become latent within the host. Lysogeny involves integration into the host chromosome.

Host
A well-known host is *Acholeplasma*.

10 PODOVIRIDAE

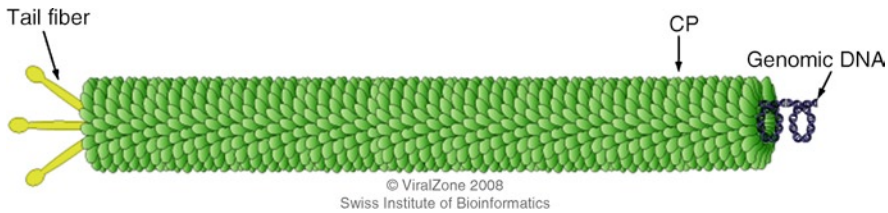


Structure
Nonenveloped, head-tail structure. Head is about 60 nm in diameter. The tail is non-contractile, has 6 short subterminal fibers. The capsid is icosahedral with a T=7 symmetry.

It has a linear, dsDNA genome of about 40-42 kb encoding for 55 genes.

Host
Wide range of bacterial hosts including Gram positive and Gram negative.

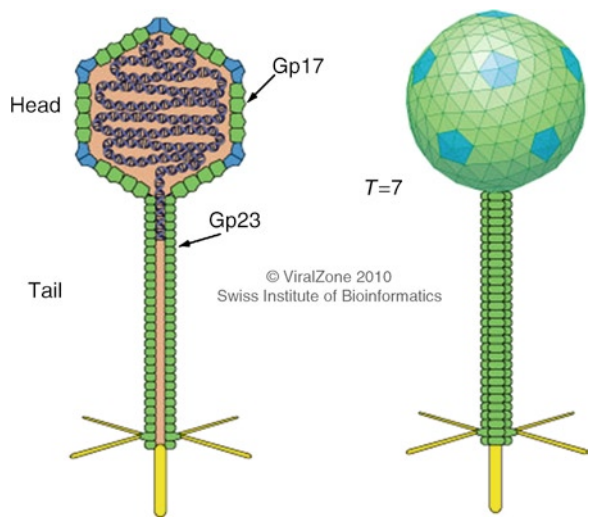
11 RUDIVIRIDAE



Structure
Non-enveloped, rod-shaped, rigid, with three tail fibers at either end. Linear dsDNA genome of 32-35 kb. At both ends, there are inverted terminal repeats as well as seven direct repeats. The two strands of the linear genomes are covalently linked.

Host
Wide range of bacteria, Gram positive and Gram negative.

12 SIPHOVIRIDAE



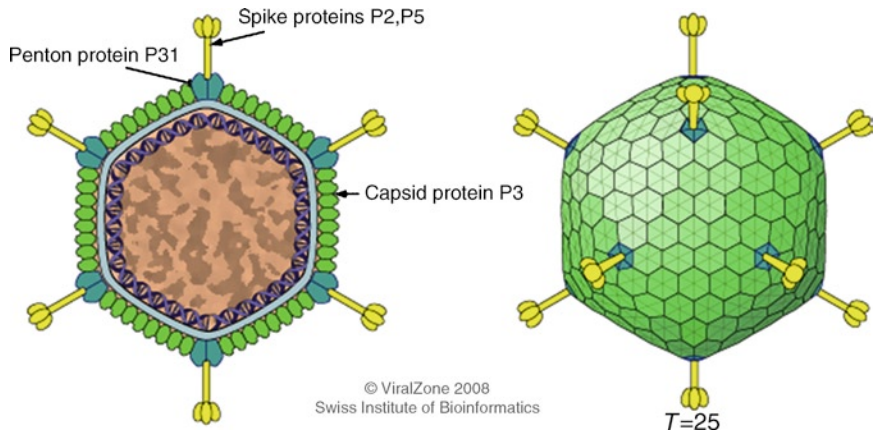
Siphoviridae are a family of double-stranded DNA viruses infecting only bacteria that are characterized by a long non-contractile tail and an isometric capsid (morphotype B1) or a prolate capsid (morphotype B2).

The *Siphoviridae* viruses have a capsid with a diameter of about 55-60 nm and a long tail that can reach up to 570 nm. Their double-stranded DNA is linear.

Nonenveloped, head-tail structure. The head is about 60 nm in diameter. The tail is non-contractile, has fibers, and is filamentous. The capsid is icosahedral with a T=7 symmetry. Linear, dsDNA genome of about 50 kb, containing about 70 genes.

Host
Wide range of bacteria, Gram positive and Gram negative.

13 TECTIVIRIDAE



Structure
Non enveloped, icosahedral virion with a pseudo T=25 symmetry. Virion size is about 66 nm with apical spikes of 20 nm. The capsid encloses an inner membrane vesicle within which the genomic DNAis coiled.

Linear, dsDNA genome of about 15 kb flanked by inverted repeats. Encodes for 30 ORFs. Replication is protein-primed.

Host
A wide range of bacteria including Alicyclobacillus, Bacillus, Enterobacteria, Pseudomonas, Thermus.

Fig. 4.28 Bacteriophages and their hosts (All items in the table reproduced with permission; Anonymous 2010d)

4.1.8.1 Crustaceans (Including Rotifers)

Microscopic crustaceans like tiny lobsters are found in sewage works where they feed on bacteria and algae. Some of the species encountered are *Cyclops* spp., *Paracyclops* spp., and *Oaphia* spp.

Rotifers, small microscopic animals of the class *Rotifera*, are found in water in great abundance. They are usually less than 1 mm, most usually in the range of 500 μm in length. Three orders are known: *Seisonidae* is marine, while the other two, *Bdelloidea* and *Monongononta*, are freshwater and found in reservoirs, streams, and sewage treatment plants.

Rotifers may be sessile or planktonic, although they can swim with their cilia; their usual locomotive method is by crawling. They are common in structures with large exposed surfaces such as in trickling filters sewage treatment plants. They are found in oligotrpic waters, i.e., waters low in organic matter, for example in sewage effluents and reservoirs after protozoa have died off, and thus are indicators of post-eutrophication waters. Indeed, they have been used as indicators of water quality. Various species are identified with different levels of water quality. *Brachionus angularis*, *Trichocerca cylindrica*, *Polyurthra euryptera*, *Pompholyx sulcata*, *Rotaria rotatoria*, *Filinia longiseta* have been designated as indicators of heavy pollution (eutrophic) while *Ascomorpha ovalis*, *Asplanchna herricki*, *Synchaeta grandis*, *Ploesoma hudsoni*, *Anuraeopsis fissa*, *Monostyla bulla,* and *M. hamata* are indicators of fresh and clean waters (oligotrophic). A variety of rotifers including *Brachionus*, *Keratella* spec, are inhabitants of moderately clean (mesotrophic) waters (Saksena 2006).

Rotifers have been also been used to detect the oocysts of *Cryptosporidium*, in water samples. The fluorescent in-situ hybridization (FISH) technique (see Sect. 4.1.3.3.2d) applied to rotifers has enabled the detection of biological contamination of surface water through an assessment of the dispersive stages of the parasite (see Table 4.19).

Other crustaceans found in water are *Daphnia*, *Cyclops*, *Synchaeta.*

4.1.8.2 Nematodes

Nematodes are invertebrate roundworms that inhabit marine, freshwater, and terrestrial environments. They comprise the phylum Nematoda (or Nemata) which includes parasites of plants and of animals,

Table 4.19 Rotifers common in aquatic environments (Modified from Saksena 2006)

S. No.	Taxonomic group	Common aquatic habitat	Food/feeding type	Examples
1.	Seisonidea	Marine epizoic	Sessile parasite	*Seison*
2.	Bdelloidiae	Freshwater planktonic, freshwater sessile, freshwater creeping	Suspension feeders	*Philodina*
3.	Monogonata	Freshwater planktonic	Suspension feeders	*Brachionus*
				Keratella
				Polyarthra
				Floscularia
			Raptorial predator	*Asplanchna*

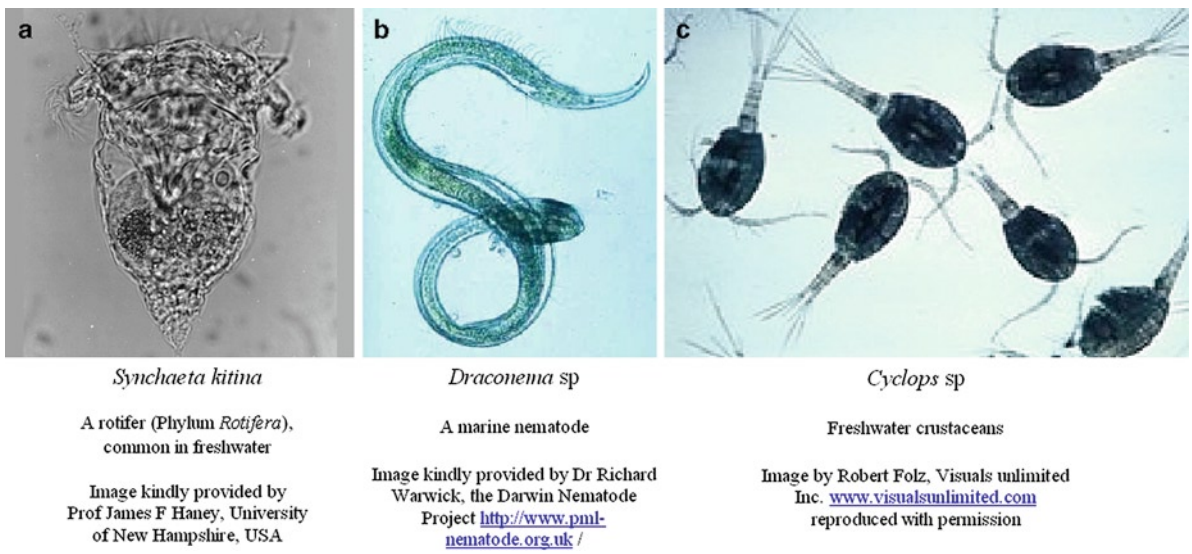


Fig. 4.29 Marine and freshwater crustaceans and nematode (All items in the table reproduced with permission)

including humans, as well as species that feed on bacteria, fungi, algae, and on other nematodes. Four out of every five multicellular animals on the planet are nematodes. The majority of nematodes are microscopic, averaging less than a millimeter in length, but some of the animal parasites are quite large and readily visible to the naked eye. Nematodes that feed on other organisms are important participants in the cycling of minerals and nutrients in the ecosystem that is fundamental to other biological activity. Some of these nematodes may have major roles in decomposition, including biodegradation of toxic compounds. In fact, the incidence of certain nematode species is sometimes used as an indicator of environmental quality (Fig. 4.29).

Nematodes are, by nature, aquatic organisms. It is estimated that about 50% of nematode species inhabit marine environments, although many of these have yet to be described and characterized. The remainder of the species inhabit soil and freshwater. In the soil, their aquatic requirements are satisfied by inhabiting the water films around soil particles. Parasitic nematodes are biologically active when bathed in moisture films supplied by water in the tissues or body fluids of the host.

Nematodes in freshwater aquatic systems also serve as a nutrient source for invertebrates, small vertebrates, and fungi. The source of food for these nematodes is primarily bacteria, but algae and fungi are also consumed.

The marine environment provides habitat for an enormous diversity of nematodes, from surface, littoral, and estuarine zones to the ocean depths. One interesting group of deep sea nematodes are the *Rhaptothyridae*, which have no mouth and a very reduced alimentary tract. The digestive tract is filled with symbiotic chemoaototrophic bacteria. A similar relationship exists in the mouthless genus *Astomonema*.

Nematodes are widely distributed in aquatic and soil habitats and are particularly common in waters rich in organic matter such as sewage. They feed on bacteria and algae etc. Some nematodes encountered are *Rhabditis* spp., *Pelodra* spp., and *Diplogaster* spp.

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