

GENERAL MICROBIOLOGY

Diversity of Microbial World

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CONTENTS

[Introduction](#)

[Taxonomic hierarchy](#)

[Binomial nomenclature](#)

[Identification and Classification Methods](#)

[Classification of Prokaryotes](#)

[Domain Archaea](#)

[Domain Eubacteria](#)

[Classification of Eukaryotes](#)

[Protozoa](#)

[Slime molds and water molds](#)

[Fungi](#)

[Algae](#)

[Viruses](#)

[Prions](#)

[Viroids](#)

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Introduction

Diversity of microbes in nature has been one of the most amazing aspects of life sciences. Microbes almost seem ubiquitous, appearing in all shapes, sizes, textures and even found in extreme environments such as at extraordinary temperatures, salinity, pressures etc. Extraordinary diversity of microorganisms makes it an absolute necessity to classify microbes in common groups based on similar properties.

Systematics

The science for studying classification is called Taxonomy (*Greek: taxis* = arrangement; *nomos* = law). Taxonomy is further divided into three working groups: Classification, Identification and Nomenclature. Classification is placing organisms within groups with members exhibiting relationships or similarities. These groups are termed as taxa (s. taxon) and the similarities can be derived from structure, physiology or evolutionary relatedness. Identification uses experimental tools to establish that a certain species belongs to a particular taxon. Nomenclature is assignment of scientific names to taxonomic groups in accordance with accepted rules. The term systematics sometimes is referred synonymously with taxonomy. While, taxonomy is plainly referred to identification, classification and naming of organisms; systematics is the evolutionary history of organisms through time.

The importance of taxonomy has been ever increasing. In 2000, a project called “All Species Inventory” was started (<http://www.all-species.org/>). The aim of the project is to identify and record every species of life by 2025. The goal is very challenging; till now 1.5 million species have been identified and it is estimated that the number of species may be between 7 to 100 million. The mind boggling number of species underscores the very importance of cataloguing the species in a proper and scientific way. Thus taxonomy is important for (i) effective communication among scientists about the identity of a particular microbe (ii) catalogue a large number of species in a systematic manner, (iii) help in predictions and further research about a particular isolate if little is known about it and it shows some similarities with microbes of particular group.

The classification or assignment of organisms to certain groups have evolved with the science and understanding. In 1735, Swedish botanist, Carolus Linnaeus introduced formal system of classification and divided organisms into *Plantae* and *Animalia* kingdoms. In 1857, Carl von Nageli placed bacteria and fungi in plant kingdom. However, Edouard Chatton in 1937 introduced the term prokaryote to distinguish cells having no nucleus from the nucleated cells of animals and plants. Interestingly, DNA sequences indicate fungi being more close to animal kingdom. In 1959, fungi were placed in separate kingdom. A separate Kingdom *Prokaryotae* was proposed by Robert G. Murray in 1968. In 1969, Robert H. Whittaker proposed five kingdom classification, where all the prokaryotes were included in the kingdom **Monera**. Eukaryotes were placed in four different kingdoms depending on their size and mode of nutrition, with the unicellular algae, protozoa and lower fungi placed in the kingdom **Protista**, photosynthetic green plants and higher algae placed in the kingdom **Plantae**, animals which ingest food were placed in the kingdom **Animalia** and organisms that absorb their food, have cell wall but lacked chlorophyll placed in the kingdom **Fungi**. Based on microscopic observations, it was assumed that there was one kind of prokaryotes. However, when sequences of the

nucleotides of ribosomal RNA (rRNA) were compared, it was observed that there were three kinds of distinct cell types; eukaryotes, bacteria (prokaryotes) and archaea (prokaryotes). In 1978, Karl R. Woese based on his studies proposed that the three cell types be elevated to a level above kingdom, and termed this level as “domain”. Figure 1 indicates the universal phylogenetic tree based on Woese suggestion. In this widely accepted division, though bacteria and archaea similar in appearance, form separate domain in evolutionary tree. Domain Eukarya encompasses animals, plants, fungi and protists. Eubacteria domain includes pathogenic as well as non pathogenic prokaryotes found in soil and water. Archaea domain includes prokaryotes found in extreme environments and do not have peptidoglycan in the cell walls.

Evolution of cell

Planet earth is estimated to be around 4.6 billion year old. Fossil remains of prokaryotic cells have been discovered from the rocks as old as 3 billion years. It is estimated that eukaryotic cells evolved 1.4 billion years ago. It was widely believed that archaea was the most primitive species. Bacteria were supposed to be more closely associated with eukaryotes. However, studies of rRNA sequence analysis indicate that universal ancestor evolved into three lineages of the Archaea, the Bacteria and the ‘modern’ nucleoplasm of the eukaryotes. According to three domain system the archaea and bacteria diverged first followed by eukaryotes. *Thermotoga maritime* is considered to have evolved before both archaea and bacteria branched away. Sequencing of this species has revealed that this microbe carry genes which are similar to both bacteria and archaea. *Thermotoga* is hence considered at the root of the evolutionary tree.

Evolution of cell organelles

Mitochondria and chloroplast in eukaryotic cell are believed to be developed later. According to endosymbiotic theory (first proposed by Lynn Margulis in 1967), at some stage in evolution prokaryotic cell invaded a primitive eukaryotic cell and provided respiratory and photosynthetic abilities previously lacking in the cell. Similarities between eukaryotic organelles (such as chloroplast and mitochondria) and prokaryotic cells are considered evidence for the endosymbiotic theory. Cyanobacteria have been widely considered as ancestors of chloroplasts. In recent times it has been shown that *Prochloron* resembles more closely with chloroplast and contains both chlorophyll a and b, but not phycobilins. Mitochondria are considered to have evolved from relationship between ancestral eukaryote and aerobic bacteria such as *Rhizobium*. In modern times, example of a prokaryote living in a eukaryote is *Cyanophora paradoxa*.

There is another view, albeit less popular, about the development of nucleus, chloroplast and mitochondria. It states that specialized organelles developed by invagination of plasma membrane to form double-membrane structures containing genetic material and capable of further development or specialization.

1. Taxonomic hierarchy

When a microbe is placed in certain group it indicates that particular species along with other members of the group have evolved in similar fashion and all the members share similar properties. Species can be termed as group of strains which share common properties but are

different from other group of strains (species). In prokaryotes taxonomy, the commonly used ranks are species, genera, families, orders, classes, and phyla (in ascending order). Microbes in each rank have a characteristic suffix particular to that rank. For example: suffix ‘-ales’ denotes Order. A collection of species constitute a **genus**, similar genera constitute **family**. A group of similar families make up an **order**, similar orders provide **class** and group of classes make up **phylum** (in botany it is called **division**). All similar phyla constitute **kingdom** and all related kingdoms make up **domain**. Following is an example how budding yeast is placed in taxonomic hierarchy.

Rank	Example
Domain	Eukarya
Kingdom	Fungi
Phylum	Ascomycota
Class	Hemiascomycetes
Order	Saccharomycetales
Family	Saccharomycetaceae
Genus	<i>Saccharomyces</i>
Species	<i>cerevisiae</i>

2. Binomial nomenclature

For millions of organisms, common names are not used as it could lead to misunderstanding as different names are used for same organism in different places. Since common names can create confusion and usually common names are in different local languages, a naming system has been introduced which is termed as “**scientific nomenclature**”. In this system every organism is given a binomial name first described by Carolus Linnaeus. The first part of Latinized name is genus which is followed by species. For example; humans are assigned scientific name as *Homo sapiens*. The scientific name is always written underlined (Homo sapiens) or italicized (*Homo sapiens*), where genus name (only first letter) starts with a capital letter. Name can be shortened by abbreviating the genus name in single capital letter (*H. sapiens*).

There are several scientific entities which assign rules for classification. Nomenclature of prokaryotes and their assignment to taxa is performed by *International Committee on Systematic Bacteriology* and published in *Bacteriological Code*. A reference called *Bergey’s Manual* lists descriptions and evidence of classification of prokaryotes. *International Code of Zoological Nomenclature* publishes rules for assigning names for parasitic worms and protozoa. Similarly rules for assigning names for fungi and algae are published in *International Code of Botanical Nomenclature*.

3. Identification and Classification Methods

There are many characteristics and techniques which are used for the identification and characterization of microorganisms. These techniques range from morphological examination to biochemical and more recent molecular biological methods.

3.1 Morphological characteristics

Morphological characterization has been one of the foremost techniques for identification and classification and also one of the first tools to help taxonomists. Morphology is easiest way to characterize complex prokaryotes and eukaryotes. The list of morphological features include cell shape, cell size, color, colonial morphology, inclusions in cell, staining pattern, motility, endospore formation and spore formation. Advances in technology, such as introduction of electron microscopy with higher resolution, have tremendously improved these studies, which were earlier solely dependent on light microscope.

3.2 Staining

Differential staining is one of the important methods employed in bacteriology. Gram stain, which is based on the composition of cell wall, can characterize bacteria into gram-positive and gram-negative groups. Another example of differential stain is acid fast stain which separates acid fast bacteria such as *Mycobacterium* (that does not decolorize with acid solution) from non-acid fast bacteria.

3.3 Source of isolation

The place from where a particular isolate has been isolated reflects microbe's ecological habitat or its host consideration. Microorganisms living in one plant may be different than other indicating host-pathogen or symbiotic relationships. Similarly microbes living in plants will be different than animals. Similarly, pattern of microflora will be different in soil, freshwater or the marine environment.

3.4 Biochemical and physiological characteristics

These tests are performed as there are hundreds of bacteria which could appear morphologically same, for example rods or cocci, yet they may differ in metabolic properties. Metabolic and physiological characteristics are indicators of the presence/absence or the expression level of microbial enzymes and proteins. Some of the biochemical and metabolic characteristics include pH, temperature and salinity requirement, carbon and nitrogen sources, fermentation products, mechanism of energy conversion, motility, oxygen requirement, photosynthetic pigments, secondary metabolites formed, sensitivity to antibiotics and inhibitors and osmotic tolerance. The methods include routine microbiological and biochemical techniques such as enzymatic tests, use of selective and differential media, growth patterns etc.

3.5 Serological characterization

Different strains among a species may carry different antigens and hence induce specific antibody response. These strains are termed as serovars or serotypes. The antisera raised against different antigens could be used for differentiation of serotypes. If antiserum raised against one species reacts with different bacterial species, both species can be tested for relatedness. The examples of serological tests include enzyme-linked immunosorbent assay (ELISA), Western blot and slide agglutination tests.

3.6 Genetic studies

Transformation and conjugation studies can be useful in characterization and finding relatedness among species. For example, *Escherichia* can conjugate with *Salmonella* and *Shigella* but not with *Proteus* and *Enterobacter* indicating *Escherichia*, *Salmonella* and *Shigella* are more closely related than the other two species.

3.7 Phage typing

There are certain viruses which infect bacteria and are called bacteriophages (or phages). A particular species of bacteria is susceptible to a certain type of phage. Phage typing refers to determining species of bacteria which is susceptible to a particular phage. This technique is used for finding similar members of a particular species and further application include tracing the origin and the progression of a disease outbreak.

3.8 Flow cytometry

A liquid sample containing suspected bacteria can be passed through a small opening in flow cytometer. The light scattering by the bacteria can be observed by detector and information about morphology is analyzed by computer interface. Antibodies raised against already identified bacteria can be used to find the relationship of a new bacterium with the existing bacteria. The bacterium in question can be incubated with antibodies tagged with fluorescent dyes. Antibodies, if specific, will bind to the surface to the bacterium to be identified and can be detected by flow cytometry. Higher the fluorescent signal detected more is the relatedness and no signal indicates lack of any relatedness among the bacterium in question and the bacterium against which the antibodies were raised.

3.9 Molecular approaches for characterization

3.9.1 DNA Composition

There are four bases of DNA, where adenine (A) pairs with thymine (T) and Guanine (G) pairs with cytosine (C). Genomes of organisms can be compared and the relatedness established based on the DNA composition. Simplest way is to determine G+C content. If G+C content is known, A+T content can be found out easily (since $100 - \text{G+C content} = \text{A+T content}$). Two closely related organisms will have similar amount of DNA base pair content and thus G+C content can be used for predictions of relatedness. However supporting data will also be required for final conclusions about similarities.

3.9.2 DNA fingerprinting

Use of restriction enzymes, which digest DNA at specific sites (the sites present at multiple sites in genome), can be used to create signatures or finger prints of a particular genomic species. DNA from two different species of microorganisms can be digested with same restriction enzyme and the fragments are separated using agarose gel. The comparison of numbers and sizes of the DNA fragments from both species give idea about the related or divergent nature of the species. Similar patterns indicate the relatedness of the species.

3.9.3 Polymerase chain reaction

Many microbes can't be cultured. However, with the advent of technique called polymerase chain reaction (PCR), it is possible to amplify the genes of the microbe and use the method for identification of new species or strains. DNA fragments obtained through PCR can be run on the agarose gel and either the presence or the absence or the size of DNA fragments can give information about the microbe. PCR in conjunction with sequencing work even better and can give definite information about the identity.

3.9.4 DNA Sequencing

- (i) **Direct DNA sequencing of the whole genomes** of microbes is best way to predict the similarities, though this method is not practical owing to cost, time factors and being labor intensive.
- (ii) **Part of the genomes can be sequenced** and compared for similarities or the differences. This approach is particularly very useful for finding variants or strains of viral or bacterial pathogens. Example: Sequencing of haemagglutinin (HA) and neuraminidase (NA) genes of the bird flu viruses helped in prediction of new strains of the virus.
- (iii) **Ribosomal RNA (rRNA) sequencing**, one of the most popular methods, is being used to determine the phylogenetic relationship among microbes. Another usage of this kind of sequencing is in determining diversity. rRNA is present in all the organisms and the sequences of rRNA appear to be conserved during evolution. The microbes, which are closely related, will have few differences in the rRNA sequences than the distantly related organisms.

3.9.5 Nucleic acid hybridization

Single stranded DNA, formed after the denaturation of corresponding double stranded DNA by heating, re-associate with complementary strands if allowed to cool below their T_m (melting temperature: the temperature at which the two strands of DNA separate from each other). When this technique is applied to separated DNA strands from two different organisms, the extent of similarity between the base sequences of the two organisms can be determined. Higher the temperatures the hybrid nucleic acid molecules can withstand, the more the two molecules are similar in sequence. This property of re-association of DNA-DNA or RNA-DNA fragments is used in hybridization techniques, such as southern and northern blotting respectively.

3.9.6 Chip technology

Chip technologies are very recent developments though currently it has limited use in phylogeny but have promising prospects. These technologies yield vast and accurate information in very short time.

- (i) **DNA Chip Technology:** An array can be made consisting of several hundred thousands of small DNA probes, where each DNA probe represents a specific species and imprinted

on a chip. DNA from unknown species is fragmented and labeled with fluorescent dye and allowed to hybridize with the DNA probes contained on the chip. Specific binding to DNA fragments on chip is detected by fluorescent reader and analyzed by computer to inform the identity of the unknown species.

- (ii) **Protein Chip Technology:** specific monoclonal antibodies to certain proteins of different species can be immobilized on a chip and protein extract from a species in question can be used for probing. Specific interaction can be detected and analyzed by the computer. This technique is currently in infancy owing to the time consuming and expensive procedure of obtaining antibodies, unlike small DNA probes used in DNA chip technology.

3.9.7 Protein sequences

Sequences of proteins are reflection of genomic status of an organism. If sequences of the proteins with same function from two microbes are similar, it reflects that both microbes are closely related. Amino acid sequences of histones, heat-shock proteins, cytochromes, and metabolic enzymes have been used for the taxonomic purposes.

4 Classification of Prokaryotes

Second edition of *Bergey's Manual of Systematic Bacteriology* contains the classification system of prokaryotes. The second edition consists of five volumes, where all volumes have yet not been published and are expected in coming years. Following table shows the contents of all the volumes of second edition of Bergey's Manual.

Volume	Contents	Features
1	Archaea and few gram negative bacteria	Includes Archaea, cyanobacteria, phototrophs, and deeply branching genera
2	Proteobacteria	Gram negative bacteria; Phylum Proteobacteria
3	Low G+C gram-positives	Phylum Firmicutes (gram positive); Phylum Mycoplasmas (wall less)
4	High G+C gram-positives	Actinomycetes
5	Chlamydiae, Spirochaetes, Bacterioidetes, Fusobacteria	Bacteria with distinct features

In new classification scheme, prokaryotes are grouped into two domains, the Archaea and the Eubacteria.

4.1 Domain Archaea

It is classified in 1st volume of second edition of Bergey's Manual. It consists of distinctive type of prokaryotic cells which lack peptidoglycan present in the cell wall of bacteria. These also share rRNA sequences which are different from those of Domain bacteria and eukaryotes. Archaea are interesting microbes characterized by highly diverse morphology and habitat. They have gained special interest among microbiologists and biotechnologists due to habitation in extreme environments of temperature, acidity and pressure. Some members of archaea are gram positive, others gram-negative while many lack cell walls. Archaea are divided into two phyla based on rRNA sequences; (i) phylum **Crenarchaeota** and (ii) phylum **Euryarchaeota**.

Phylum **Crenarchaeota** is divided into orders Thermoproteales, Desulfurococcales and Sulfolobales. The members of these orders are thermophilic and hyperthermophilic sulfur-metabolizing archaea.

Phylum **Euryarchaeota** contains methanogenic, halophilic, thermophilic and sulfur reducing prokaryotes.

Important groups of Archaea based on physical properties and habitats are given below:

Halophiles: These are major group of archaea, living in very high concentration of salts. Examples include *Halobacterium* and *Halococcus* which live in high concentration of sodium chloride and require such concentration of salt for survival.

Sulfur-metabolizing archaea: These are present in acidic, sulfur rich hot springs. Example includes *Sulfolobus*, which thrives at a pH range of 2 and temperatures of more than 70°C. Archaea of these types are also present in deep ocean trenches near hydrothermal vents.

Methanogenic archaea: These are obligate anaerobes which produce methane by combining hydrogen with carbon dioxide. Examples include *Methanobacterium*, *Methanosarcina* and *Methanospirillum*. These organisms have found great importance for the treatment of sewage under anaerobic conditions.

Thermoplasmas: These organisms lack cell wall and grow at high temperature (55 to 59°C) and low pH (pH2) and have been isolated from piles of burning coal refuse.

4.2 Domain Eubacteria

As shown in table based on Bergey's Manual, Eubacteria have been divided into five groups (or volumes). Following is given account of domain bacteria from each volume.

4.2.1 First volume of Bergey's Manual

Along with Archaea, 1st volume of Bergey's manual also contains few of the gram negative bacteria including cyanobacteria, phototrophs, and deeply branching microbes. Following are some of the important phyla:

- (i) Phylum **Aquificae**: Members use hydrogen to reduce oxygen thereby producing energy and water. Example: *Aquifex*.
- (ii) Phylum **Deinococcus Thermus**: Members contain high concentration of carotenoid pigments which makes them resistant to radiations. Example: *Dienococcus*.
- (iii) Phylum **Chloroflexi**: Members are called green non-sulfur bacteria. Examples include *Chloroflexus* and *Herpetosiphon*. These bacteria lack lipopolysaccharides in outer membrane.
- (iv) Phylum **Chlorobi**: Members contain anoxygenic photosynthetic bacteria known as green sulfur bacteria. Members oxidize sulfide to sulfur granules. Example: *Chlorobium*.
- (v) Phylum **Cyanobacteria**: Members contain oxygenic photosynthetic bacteria. Cyanobacteria contain chlorophyll a and phycobilins. These bacteria incorporate carbon dioxide just like plants through the Calvin cycle. Morphologically these bacteria may appear unicellular or filamentous, branched or unbranched. Example: *Nostoc*, *Oscillatoria*

4.2.2 Second Volume of Bergey's Manual

The second volume of the Bergey's Manual contains gram negative phylum **Proteobacteria**, also called purple bacteria. Purple bacteria in second edition have been phylogenetically grouped based on 16S rRNA sequence comparisons unlike phenotypic properties as was done for the first edition. The bacteria in this phylum are very diverse and range from obligate intracellular parasites to independent living habitat in water and soil. The name of the phylum is based of Greek god "Proteus", which could take many shapes; indeed **Proteobacteria** which are largest taxonomic group contain bacteria not only with simple morphology but also with stalks, buds, prostheca and, sheaths. These bacteria can also be phototrophs, heterotrophs and chemolithotrophs. The well known genera from this phylum include *Escherichia*, *Pseudomonas*, *Rhizobium*, *Vibrio*, *Salmonella* etc which shows the importance of the phylum in human health, research and biotechnology. Since many of the genera contain photosynthetic bacteria, it is believed that the phylum evolved from a common photosynthetic ancestor. The phylum is divided into five classes based on 16S rRNA sequence data:

Class I – Alphaproteobacteria (α)

Alphaproteobacteria includes most of proteobacteria which are capable of growing at very low levels of nutrients. Some of the members have unusual morphology such as stalk and prosthecae. Also some of the plant and human pathogens as well as symbionts are members of Alphaproteobacteria. This class has six orders and 18 families. Important members are discussed below:

Rickettsia: These are rod shaped obligatory pathogenic bacteria transmitted to humans by tick and insect bites. *Rickettsia rickettsii* is responsible for Rocky Mountain spotted fever, while *R. typhi* and *R. prowazekii* are causative agents for the typhus fever.

Caulobacter and Hyphomicrobium: Species in the genus *Caulobacter* contain prostheca. These are usually isolated from freshwater as well as marine habitats. These bacteria carry stalk that attaches the cell to surfaces. Stalk helps to take nutrients from other bacteria and algae. Members of genus *Hyphomicrobium* are budding, aerobic bacteria which are attached to surfaces through prostheca.

Acetobacter and Gluconobacter: These bacteria convert ethanol into acetic acid and hence are of considerable industrial value.

Rhizobium: These are symbiotic bacteria which infect the roots of leguminous plants. The bacteria induce nodule formation on the roots of infected plants and help in fixing the atmospheric nitrogen for the plants.

Agrobacterium: These bacteria induce a disease called ‘crown gall’ near the junction of roots and stem. Important species of this genus *A. tumefaciens* contains a plasmid, a part of which (T-DNA) gets integrated into the plant genome during the infection. This property of *A. tumefaciens* has been exploited by biotechnologists for research.

Nitrobacter and Nitrosomas : These are genera of nitrifying bacteria . *Nitrobacter* oxidizes nitrite to nitrate, while *Nitrosomas* oxidizes ammonia to nitrite. When both *Nitrobacter* and *Nitrosomas* grow together, these can convert ammonia to nitrate in the process called nitrification.

Azospirillum: *Azospirillum* grows in close association with roots of many plants and in exchange for excreted nutrients from plants fixes nitrogen from air for the plant. This genus is found in close association with plants such as tropical grasses and sugarcane and is of interest to plant biotechnologists.

Brucella: Members of this genus are obligate parasites of mammals and cause disease called brucellosis.

Wolbachia: These bacteria live inside the body of host (insects); in a relationship called endosymbiosis.

Class II – Betaproteobacteria (β)

The properties of the members of this class overlap with those of alphaproteobacteria. Members of Betaproteobacteria mostly grow on the leached nutrients from the anaerobic habitats. Some of the important pathogenic members are also part of this group. Important members are discussed below:

Spirillum: These bacteria are isolated from fresh water habitat and have bipolar flagella for motility as special feature.

Sphaerotilus: *Sphaerotilus* is found in fresh water and sewage. *Sphaerotilus* forms a long hollow sheath around it for protection and nutrient uptake. One of the species *S. natans* is commonly known as sewage fungus.

Leptothrix: This is also a sheathed bacteria and deposit oxides of iron and manganese in its sheath. This helps in growth of *Leptothrix* in presence of high concentration of soluble iron.

Neisseria: These are non motile, aerobic cocci, which occur in mucous membranes of mammals. Among pathogens, *Neisseria gonorrhoeae* the causative agent for gonorrhea and *N. meningitidis* the agent responsible for meningococcal meningitis have derived most attention.

Bordetella: These are non motile, aerobic rods. *Bordetella pertussis* is causative agent for pertussis or whooping cough, an important human disease.

Thiobacillus: *Thiobacillus* is a colorless sulfur bacterium, oxidizes sulfur and is important for sulfur cycle. Bacteria like *T. ferrooxidans* thrives in sulphuric acid containing habitats. Production of sulfuric acid and ferric iron by *T. ferrooxidans* leads to a major pollution problem called 'acid mine drainage' and can cause extensive damage to fauna and flora of natural water bodies.

Burkholderia: Recently *Pseudomonas* was divided into seven new genera based on rRNA data; where *Burkholderia* being one of the genera is now placed in Betaproteobacteria. *Burkholderia* are motile with single or many polar flagella. One of most important species *B. cepacia*, can degrade many of the organic molecules and can help in recycling in nature. *B. cepacia* is also a problem in hospitals in the contamination of drugs and equipments as it can even grow in disinfectants. This species is also found to be problematic in patients of cystic fibrosis.

Zoogloea: These bacteria form characteristic fluffy, slimy masses during growth. This kind of growth is required in the sewage treatment methods and makes this genus industrially important.

Class III – Gammaproteobacteria (γ)

Gammaproteobacteria represents the largest subgroup of proteobacteria and is characterized by the exceptional diversity as evident by 14 orders and 25 families. The main orders and respective type genera are discussed below:

Order: Chromatiales

Purple sulfur bacteria: These are photolithoautotrophs and strict anaerobes. These bacteria oxidize hydrogen sulfide to sulfur. *Thiospirillum* and *Chromatium* are typical purple sulfur bacteria and are found in anaerobic, sulfide- rich zones of lakes.

Order: Thiotrichales

Beggiatoa: *Beggiatoa* is microaerophilic and grows in sulfide-rich habitats. Morphologically it resembles filamentous cyanobacteria but is not photosynthetic. Its only species is *B. alba*.

Francisella: This genus consists of pleomorphic, small bacteria which grow on media rich in blood. *F. tularensis* causes the disease tularemia.

Order: Pseudomonadales

Members of the order Pseudomonadales are aerobic, gram-negative rods or cocci.

Pseudomonas: This is an important genus of rod shaped bacteria, abundant in soil and natural environments. These are motile by polar flagella, which can be single or in tufts. These bacteria are aerobic in nature and in second edition of Bergey's manual many members of this group have been placed in many newly named genera such as *Burkholderia*. *P. aeruginosa* infects people with low immunity, with burn wounds and can cause sepsis, urinary tract infection and even meningitis. It can also infect patients suffering from cystic fibrosis. Pseudomonads in general account for the ten percent of the hospital acquired infections. Their ability to grow in trace amounts of unusual carbon sources, even in some of the antiseptics and resistance to most of the antibiotics is a serious medical concern. Genome of *P. aeruginosa* has been sequenced and in comparison to *E. coli* has been found to be much more complex and carry larger number of genes. Other species of *Pseudomonas* such as *P. syringae* and *P. cepacia* are plant pathogens. Many Pseudomonads can grow at lower temperatures and are responsible for food spoilage. Some Pseudomonads such as *P. aeruginosa*, *P. syringae*, *P. putida* and *P. fluorescens* are placed in fluorescent subgroup. Fluorescent subgroup produces water soluble, yellow green pigment which fluoresces under UV radiation.

Azotobacter: Species of this genus are widespread in water and soil. These are large, ovoid bacteria and can form cysts. Important property includes fixation of nitrogen nonsymbiotically.

Moraxella: Members of this genus are aerobic coccobacilli. One species, *M. lacunata*, is a pathogen and is responsible for conjunctivitis.

Order: Vibrionales

Members of this order are facultatively anaerobic found in aquatic habitats. The shape of bacteria is rod like with slight curve. Vibrionales contains only one family *Vibrionaceae* with six genera. *Vibrio* is most important genera of *Vibrionaceae*, where one of the species *V. cholerae* is the causative organism of the disease cholera. Cholera involves severe watery diarrhea. Another species *V. parahaemolyticus* causes gastroenteritis, which is not a serious disease as cholera. *V. fischeri* lives symbiotically in luminous organs of fishes. *V. fischeri* is capable of bioluminescence and emit blue green light through the activity of enzyme luciferase.

Order: Legionellales

Legionella: This genus has been associated with a pneumonia called legionellosis. These bacteria though found in streams can grow in air conditioning system and water supply lines thus creating concerns of sporadic outbreaks.

Coxiella: *C. burnetti* is a pathogen and associated with Q fever.

Order: Enterobacteriales

Members of this order are facultative anaerobes, gram-negative, peritrichously flagellated or non-motile, rods with simple nutritional requirements. These are also called 'enterics' reflecting the fact that these bacteria inhabit intestinal tracts of humans and animals. Most of these ferment glucose and other carbohydrates.

Escherichia: *E. coli* is one of the most known organism in microbiology. *E. coli* is also one of the major habitants of human intestinal tract. Generally considered harmless flora, it can occasionally lead to urinary tract infections and certain strains can produce traveler's diarrhea

and food-borne disease. Presence of *E. coli* in water is an indication of fecal contamination. Researchers consider this species as 'laboratory pet' and it continues to be an important tool for biotechnological research and development.

Salmonella: Most species of this genus are pathogenic. *Salmonella* are common inhabitants of the intestinal tracts of cattle and poultry and can contaminate food. *S. typhi* is responsible for typhoid fever, the most serious illness caused by Salmonellas. Another disease by *Salmonella* called salmonellosis is characterized by severe gastrointestinal disorder.

Shigella: Unlike *Salmonella*, species of *Shigella* are only present in humans. They are responsible for shigellosis or bacillary dysentery. They are also responsible for traveler's diarrhea.

Klebsiella: The species of *Klebsiella* are found in water and soil and some of the isolates can fix nitrogen. *K. pneumoniae* can cause pneumonia in humans.

Enterobacter: *E. cloacae* and *E. aerogenes* are responsible for urinary tract infections.

Yersinia: *Y. pestis* causes plague; the dreaded disease from ancient times. Mode of transmission is through fleas which can transmit bacteria among animals (rats, squirrels) and humans.

Serratia: *S. marcescens* produces characteristic red pigment and is responsible for respiratory and urinary tract infections acquired from hospitals.

Proteus: Members of this genus causes urinary tract infections. On agar, colonies of *Proteus* show characteristic swarming movement.

Erwinia: Members of this genus are mainly plant pathogens and cause plant rot.

Order: Pasteurellales

Members of this order are small and non-motile. Members are best known for the diseases they cause in humans and animals. There is only one family *Pasteurellaceae* which carry six genera.

Pasteurella: Members of this genus mainly causes disease in domestic animals. *P. multilocida* can be transmitted to humans by dog and cat bite. *P. haemolytica* is responsible for 'shipping fever' in cattles.

Haemophilus: This genus contains pathogenic bacteria. Bacteria require blood in the medium for growth. *H. influenzae* type b is responsible for meningitis in children. *H. ducreyi* causes sexually transmitted disease chancroid.

Class IV – Deltaproteobacteria (δ)

This class has been divided into seven orders and 18 families. There are two groups of bacteria, one type are predators on other bacteria whereas second type are involved in sulfur cycle.

Order: Bdellovibrionales

This order contains only one family *Bdellovibrionaceae*. Genus *Bdellovibrio* is aerobic, curved rod with long polar flagella. *Bdellovibrio* infects other gram-negative bacteria and multiply in the periplasm. *Bdellovibrio* while growing on cells it infects, produces plaques on the culture plates just as bacteriophages.

Order: Myxococcales

Members are aerobic, present in soil and move by characteristic gliding. They have interesting life cycle where bacteria produce fruiting bodies and dormant myxospores. Most members of Myxococcales are micropredators and secrete enzymes that lyse their prey such as other bacteria and yeasts. *Myxococcus* is the type genus for this order.

Order: Desulfovibrionales

Members of this order are sulfur reducing bacteria. These are obligate anaerobes which use sulfates and elemental sulfur, instead of oxygen as electron acceptor and produce H_2S . These bacteria play a key role in the sulfur cycle by releasing millions of tons of H_2S into the atmosphere every year which in turn is utilized by sulfur-oxidizing bacteria such as *Beggiatoa* as part of photosynthesis or as an autotrophic energy source. Best studied genus of this order is *Desulfovibrio*.

Class V – Epsilonproteobacteria (ε)

This is the smallest class among proteobacteria and consists of only one order Campylobacterales and three families. There are two important genera *Campylobacter* and *Helicobacter* which are microaerophilic and motile by flagella.

Campylobacter: One of the species of this genus, *C. fetus* is responsible for spontaneous abortions in animals. Another important species *C. jejuni* causes enteritis and diarrhea in humans.

Helicobacter: Most important species of this genus, *H. pylori* is major human pathogen and causes gastritis and peptic ulcer disease. It is estimated that more than 50% of world population is infected with this pathogen, though precise mode of transmission is unclear. Genomes of *H. pylori* as well as *C. jejuni* have been sequenced.

4.2.3 Third Volume of Bergey's Manual

This volume consists of gram-positive bacteria which have low G+C content. These are assigned one phylum i.e. Firmicutes. This phylum includes genera of medical importance, bacteria which forms endospore, bacteria with industrial importance as well mycoplasma which lack cell wall. Important orders are discussed below:

Order: Clostridiales

Clostridium: These endospore forming rod-shaped bacteria are obligate anaerobes. Endospores produced by this genera are resistant to heat and other modes of sterilization and hence species of *Clostridium* are of considerable importance for food and health industry. Diseases caused by species of *Clostridium* include botulism (caused by *C. botulinum*), gas gangrene (caused by *C.*

perfringens) and tetanus (caused by *C. tetani*). *C. pasteurianum* is another important species which is responsible for anaerobic nitrogen fixation in the soil.

***Epulopiscium*:** The type species of this genus *E. fishelsoni* is a giant bacterium with size as large as 80 μm x 600 μm . This bacterium can be seen though naked eye and carry 25 times more DNA than a human cell. Recently another giant bacterium (bigger than *E. fishelsoni*) *Thiomargarita namibiensis* has been reported which has a size of 750 μm in diameter. However, *T. namibiensis* is not related to *Epulopiscium* and has been placed in gamma-proteobacteria.

Order: Bacillales

***Bacillus*:** Species of this rod shaped genus are also involved in endospore formation. *B. thuringiensis* and *B. popilliae* are insect pathogens and spore produced by these species are used as insecticides. *B. anthracis* is responsible for anthrax, a disease of cattle which can be transmitted to humans. Recently it has gained negative importance as an agent for biological warfare and terrorism.

***Staphylococcus*:** Species of *Staphylococcus* are commensals and occasionally cause serious infections in humans. Important species *S. aureus* can result in serious hospital acquired infection, because of the ability to develop resistance to standard antibiotics. *S. aureus* produces toxin which is responsible for toxic shock syndrome. Another species *S. epidermidis* found on skin is non pathogenic

Order: Lactobacillales

***Lactobacillus*:** *Lactobacillus* is representing genus of Lactobacillales and is a commensal of humans. Species of *Lactobacillus* produces lactic acid and are industrially important bacteria employed in food industry for making of products such as cheese, pickles and yogurt. Example: *L. delbrueckii* is employed for production of yogurt.

***Streptococcus*:** Few species of this genus have importance in food industry and are employed in the production of fermented products. Many of the species of *Streptococcus* are human pathogens. *S. pyogenes* causes scarlet fever and rheumatic fever.

***Listeria*:** One species of this genus, *L. monocytogenes* causes important food borne disease, listeriosis.

Order: Mycoplasmatales

Mycoplasmas lack cell wall and hence are pleomorphic. These organisms are of considerable evolutionary importance being smallest replicating organisms capable of host free existence. These organisms though do not stain as gram-positive yet are phylogenetically related to gram positive bacteria with low G+C content. Mycoplasmas produce hyphae like growth resembling fungi hence initially derived the name (myco = fungus). Species of Genus *Mycoplasma* are parasites of animals and plants. *M. pneumoniae* is responsible for pneumonia. Species of another genus *Spiroplasma* are pathogens of plants and insects.

4.2.4 Fourth Volume of Bergey's Manual

Fourth volume consists of phylum Actinobacteria comprising of gram-positive bacteria having high G+C content. Except *Mycobacterium tuberculosis* all members are commensals. Some members of the group are of major economic importance because of the potential for antibiotics and fermented dairy products.

Corynebacterium: These are non-motile and appear as club shaped under the microscope (koryne = club). One species, *C. diphtheriae* is the causative organism for diphtheria.

Arthrobacter: Species of this genus are among most common of the soil bacteria. They are resistant to various modes of sterilization and starvation despite not having the ability to form spores.

Propionibacterium: The name of the genus is derived because these bacteria can produce large amounts of propionic acid. Some species are important for making swiss cheese while one of the species, *P. acne*, which is commonly found on human skin, has been implicated in forming acne.

Mycobacterium: These are rod shaped and have a unique staining ability called acid-fastness owing to presence of special lipids called mycolic acids on the bacterial surfaces. A number of species are found in soil. Human pathogens of concern include *M. tuberculosis* (causes tuberculosis) and *M. Leprae* (causes 'leprosy).

Bifidobacterium: These are obligate anaerobes with coryneform cells, commonly found in the intestinal tract of breast-fed infants.

Frankia: This bacterium forms nodules in the roots of angiosperms for symbiotic nitrogen fixation.

Streptomyces: This genus contains a large number of species (more than 500) and strains which are mostly found in soil. The colonies of these species form filaments called sporophores which give rise to spores called conidia. These sporophores and conidia are pigmented and give distinct color to the colony. The special earthy smell of the earth is caused by geosmins which are products of *Streptomyces* metabolism. Species of *Streptomyces* are very important medically as they are source of most of available antibiotics.

Actinomyces: Species of this genus are facultative anaerobes found in humans and animals. *A. israelii* causes actinomycosis.

Nocardia: The species are found in soil and can give acid fast stain. One species *N. asteroides* is an occasional pathogen causing pulmonary infection.

4.2.5 Fifth Volume of Bergey's Manual

Fifth volume of Bergey's Manual consists of phyla such as Chlamydiae, Spirochaetes and Bacteroidetes.

Phylum: Chlamydiae

The members do not contain peptidoglycan in the cell wall.

Chlamydia: Species of *Chlamydia* are gram-negative bacteria which are obligate parasites and cause variety of diseases in humans. *C. psittaci* is responsible for psittacosis; an epidemic disease of birds which can be transmitted to humans and causes mild form of pneumonia. *C. trachomatis* leads to trachoma, main cause of blindness in humans. Other strains of *C. trachomatis* are responsible for genitourinary infection and are leading causes of chlamydial urethritis and lymphogranuloma venereum. *C. pneumoniae* is cause of mild forms of respiratory syndromes.

Phylum: Spirochaetes

Members are motile, gram-negative, and tightly coiled bacteria. These bacteria are found in aquatic habitats and in animals. Motility is by periplasmic flagella, which may be one to few in numbers and emerge from each pole.

Treponema: One of species, *T. pallidum* is cause of sexually transmitted disease syphilis.

Borrelia: Species of this genus causes relapsing fever in humans and borreliosis in cattles.

Leptospira: *Leptospira* causes leptospirosis through contaminated water.

Brachyspira: *Brachyspira* causes diarrhea in chicken and swine.

Phylum: Bacteroidetes

Bacteroides: This group of anaerobic bacteria synthesizes a characteristic type of lipid called sphingolipids. These are normal commensals found in intestines of humans and animals. According to estimates over one billion bacteria can be found in one gram of human feces. These species can also be pathogens and have been found in wounds and surgical infections.

Flavobacterium: One species *F. meningosepticum* is a pathogen and is responsible for infant meningitis.

Cytophaga: These gram-negative bacteria move by gliding. Species of *Cytophaga* are important in the digestion of cellulose and chitin.

5. Classification of Eukaryotes (Domain Eukarya)

In context of microbial phylogeny, members of *eukarya* form a domain which is phylogenetically more close to *archaea* than to *eubacteria*. There is agreement on the classification system of *archaea* and *eubacteria* based on the 16S rRNA sequence comparison. In the case of *eukarya*, this system is based on the comparative sequencing of 18S ribosomal RNA from the cytoplasmic ribosomes. Following is the description of microbes in Eukarya:

5.1 Protozoa

These are unicellular eukaryotic microbes which are found in water and soil. Protozoa lack cell wall and are motile. They differ from algae by lack of chlorophyll and from fungi by having the property of motility and absence of cell wall. Many species of protozoa are parasites of other animals and humans. Most of the species feed by surrounding the food with portion of the membrane and intake the food by a process called phagocytosis. Though protozoa have been classified into several phyla based on rRNA sequencing, we will discuss protozoa based on taxonomic subgroups arrived on the basis of one of the main physical characteristic, i.e. motility. Based on motility protozoa have been divided in four sub-groups; *Apicomplexa* (non-motile), *Sarcodina* (move by ameboid motion), *Mastigophora* (move by flagella) and *Ciliophora* (move by cilia).

5.1.1 Apicomplexa

These are also called sporozoans and are obligate parasites in nature. Members contain special organelles which carry enzymes for tissue penetration at the tip (or apex) of the cells. Life cycle of these protozoa is complex and involves transmission among several hosts. The term sporozoan is derived from 'sporozoites' which is one of the forms of protozoan assumed during the transmission to different hosts. The members from Apicomplexa are involved in several diseases such as malaria (*Plasmodium* species), which is one of the major human diseases and toxoplasmosis (*Toxoplasma* species).

5.1.2 Sarcodina

Members move by extending lobe like structures called pseudopods. *Amoeba* is one of the common species found in freshwater.

***Foraminifera*:** The species are found in marine water and have special structure outside called shells. Shells are not firmly attached to the amoeba cell and during feeding cell can leave the shell to some distance and come back. Shells are made up of calcium carbonate, are resistant to decay and can become fossilized.

***Entamoeba histolytica*:** This protozoan is human pathogen and can cause amoebiasis characterized by dysentery. This pathogen can be transmitted from human to human through oral fecal route.

5.1.3 Mastigophora

The members are motile by flagella and hence are also called flagellates. Most species are found in freshwater and some are parasites.

***Trypanosomes*:** This is an important pathogenic genus which causes several serious diseases in humans and animals. These are thin crescent shaped microbes. *T. brucei* is responsible for African sleeping sickness. The organism is transmitted from human to human by blood sucking tsetse fly.

Trichomonas: One of the species, *T. vaginalis*, is a pathogen and is responsible for urethritis and vaginitis. This protozoan is found in vagina and male urinary tract.

Euglena: This genus is one of the few phototrophic protozoa. *Euglena* contains chloroplasts which allow photosynthetic growth, though in dark the cells become chemo-organotrophs. The species dwell in freshwater and are non pathogenic.

5.1.4 Ciliophora

The members are called ciliates because at some stage of the life cycle they possess cilia, which is required for motility. Ciliates have two characteristic nuclei; micronucleus (required for sexual reproduction and inheritance) and macronucleus (required for transcription and cell functions).

Paramecium: This is best known genus of ciliates, obtain its food by ingestion through oral region. When food is trapped inside vacuole, digestive enzymes are secreted and break down the food. Some species of *Paramecium* harbor endosymbiotic bacteria, which synthesize nutrients for the protozoan, in the cytoplasm.

Balantidium: *Balantidium* is probably the only genus of ciliates which is pathogenic for human. Species *B. coli* is responsible for balantidial dysentery and is transmitted through oral fecal route.

5.2 Slime molds and water molds

Slime molds are eukaryotic microbes similar to both protozoa (amoebal features and motility) and fungi (have life cycles and ability to produce spores) but are phylogenetically distinct.

5.2.1 Acrasiomycota (Cellular slime molds)

These are single cells which resemble amoeba. Amoeboid cells ingest bacteria and fungi by phagocytosis. *Dictyostelium discoideum* is widely studied species of cellular slime molds. This is model organism for scientists who are working on cellular migration, aggregation and cellular communication in microorganisms. When conditions are unfavorable, such as starvation, the large number of cells of *Dictyostelium* aggregate to form a single slimy mass called 'slug', where though cells are not completely independent but they do not fuse or break. The aggregation is brought about by the release of cAMP by some of the cells. When slug stops migration, differentiation happens, fruiting body is formed and ultimately spores are produced. Spores germinate and give rise to amoebic cells.

5.2.2 Myxomycota (Acellular or plasmodial slime molds)

Acellular slime molds are mass of expanding and non-defined cytoplasm with many nuclei. The mass of cytoplasm with many nuclei is called plasmodium. The genetic content in plasmodium is diploid and a sporangium can be produced from plasmodium. Sporangium gives rise to haploid spores, which in turn in favorable situations produce swarm cells. Two swarm cells can fuse and produce diploid plasmodium. The movement of plasmodium is through a mechanism called 'cytoplasmic streaming'.

5.2.3 Oomycota

These are also commonly called as water molds and are found in aquatic habitats. The sexual spore type is oospore and motile cells of these fungi are characterized by two lateral flagella, one is of tinsel type while other is a whiplash flagella. Members of this group are either saprophytic or parasitic for example *Phytophthora infestans* which causes late blight disease of potato and led to massive economic losses during 19th century in Germany and Ireland.

5.3 Fungi

Fungi differ from protozoa in terms of having cell walls and producing spores, while from algae in terms of not able to perform photosynthesis. The cell wall of fungi is composed of chitin. Fungi are important microbes with many industrial, ecological and medicinal benefits and have been implicated in the important diseases of plants, animals and humans. Fungal diseases such as *Candida albicans* infection and *Pneumocystis carinii* mediated pneumonia in AIDS patients have attracted lot of medical attention in recent times. Mushrooms are used as food, while other fungi such as yeasts are used for making food (bread and alcoholic beverages), molds are required to produce antibiotics and many others are invaluable for the ability to fix minerals in higher plants in a symbiotic relationship called mycorrhizae. Most fungi are terrestrial though some genera are found in freshwater as well as marine habitats. Fungi follow absorptive mode of food acquisition method, have simple nutritional requirements and are chemoheterotrophs in nature. Ability of some fungi to grow at environmental extremes makes them contaminants of concern. Unicellular fungi (yeasts) are identified by biochemical tests whereas multicellular fungi (molds, mushrooms) are characterized by physical appearances such as colony characteristics and spores types.

5.3.1 Molds

These are filamentous fungi and the filament is called hypha (plural: hyphae). Hyphae grow together and form compact mass called mycelium. In most molds, hyphae are septate, i.e. the cells are separated by cell walls. In some fungi there are no septa and cells appear continuous and have multinucleate morphology, these types of hyphae are called coenocytic hyphae. Fungal mycelium may have aerial branches which bear asexual spores called 'conidia'. Molds also produce sexual spores from the fusion of nuclei from two different mating types of same species. Different types of phylogenetic groups produce different types of sexual spores. Spores formed within a sac like structure are called "ascospores" (ascus: sac). "Basidiospores" are produced at the end of a club like structure (basidium). "Zygospores" are large sexual spores surrounded by thick wall like structure. Both sexual as well as asexual spores can germinate and give rise to new mycelium.

5.3.2 Mushrooms

These are macroscopic basidiomycetes where large characteristic fruiting bodies are formed (mushrooms). Normally the fungus grow as simple mycelium in soil and plant litter but under favorable conditions well shaped round fruiting bodies are formed which reach above ground.

Basidiospores are formed underside of fruiting bodies and in favorable conditions germinate and give rise to mycelium.

5.3.3 Yeasts

Yeasts are unicellular, non-filamentous fungi and most members belong to ascomycetes. The cell division of yeasts mostly takes place by budding. In budding yeast, for example, *Saccharomyces*, parent cell form a bud outside its surface, the bud elongates and after nuclear division, the nucleus enters into it and elongated bud pinch off as new cell. Yeasts are capable of facultative anaerobic growth. In the presence of oxygen they metabolize carbohydrates to carbon dioxide and water. In the absence of oxygen, they ferment carbohydrates and produce ethanol and carbon dioxide. Species of *Saccharomyces* are used in food industry for making bread and ethanol. The human pathogen *Candida albicans* is also yeast but forms pseudohyphae required for the invasion in the host cells. There are also fission yeasts such as species of *Schizosaccharomyces* which divide into two equal halves during cell division.

5.3.4 Short account of classification of Fungi

Fungi are divided into five groups based on differences in thallus organization and sexual reproduction.

Division: Chytridiomycota

These are the simplest true fungi, commonly known as chytrides. They produce motile asexual spores called zoospores, possessing a single, posteriorly positioned whiplash flagellum. Members of this class are either parasitic on algae or grow saprophytically on dead organic matter. Example: *Allomyces macrogynus*

The Terrestrial Fungi are characterized by a complete lack of motile cells. There are four major groups as discussed below:

Division: Zygomycota

These are commonly referred as bread molds. The mycelium is composed of coenocytic hyphae and sexual spores are of zygospore type hence the name of the group. Species are found in soil and on plant litter. Species of *Mucor* and *Rhizopus* (common bread mold) are involved in food spoilage; however, sometimes both of these genera being opportunistic pathogens in patients with diabetes mellitus and leukemia can cause systemic mycosis.

Division: Ascomycota

Members of ascomycetes are also referred to as sac fungi and are found in soil and decaying plant material. They produce spores during sexual cycle which are called ascospores. The hypha produced is of septate type. The important diseases of plants caused by members of Ascomycetes include Dutch elm disease, chestnut blight, rots and ergots. Some species are also human pathogens such as *Histoplasma capsulatum* and *Blastomyces dermatidis* causing different

forms of systemic mycosis. *Neurospora*, a member of Ascomycetes, is used as a model fungus for studying genetics.

Division: Basidiomycota

These contain septate hyphae and are found in soil and decaying plant materials. The name of group is derived from the presence of sexual spore basidiospores in the life cycle. Club fungi and Mushrooms are part of Basidiomycetes. Important genera of mushrooms include *Amanita* (poisonous mushroom) and *Agaricus* (edible mushroom). Members of Basidiomycetes are involved in ecological activities and play crucial role in the decomposition of wood, paper and other materials derived from the natural sources. Some genera called 'wood rotting fungi' are exclusively involved in degradation of lignin in nature. Some of the plant diseases caused by fungi of Basidiomycetes include wheat rust and corn smut. *Cryptococcus neoformans* is human pathogen, acquired through inhalation and causes systemic mycosis.

Division: Deuteromycota

These are also called fungi imperfecti, because they either lack sexual reproduction or their sexual stage is not yet discovered. Deuteromycetes fungi have septate mycelium. The type members include medically important genera of *Penicillium*, *Aspergillus* and *Candida*. Some species of *Penicillium* and *Aspergillus* are now placed under Ascomycetes as their sexual stages have been discovered. Once the sexual stage is discovered and the fungi is moved from Deuteromycetes to Asco- or Basidiomycetes depending on the type of sexual spores it produces and its name is also changed for example *Penicillium* have been renamed as the ascomycetes genus *Talaromyces*. The members of fungi imperfecti are found in soil, on animal bodies and decaying plant litter. *Aspergillus niger* is of great commercial importance and used to produce citric acid and amylase. Plant diseases caused by genera of Deuteromycetes include wilts, while human diseases include diseases such as candidiasis by *Candida albicans*, aspergillosis by species of *Aspergillus*, ringworm infection by *Microsporum* and athlete's foot by *Trichophyton*.

5.4 Algae

Algae are eukaryotic organism which contain chlorophyll and carry out photosynthesis. A small number of algae are multicellular and macroscopic; however, most of the genera of algae being microscopic and unicellular, algae are usually studied with other microorganisms. Algae are found in oceans and in aquatic habitats. The cells contain chlorophyll and appear green in color. However, certain types of algae contain pigments such as xanthophylls and carotenoids, which make algae appear as brown or red, as such pigments mask the color of the chlorophyll. The cells also contain one or more chloroplasts, the site for photosynthesis. All algae divide asexually; unicellular by binary fission and filamentous forms just simply fragment and each fragment than is capable of forming filamentous forms again.

Algae are classified based on 18S rRNA sequencing comparisons. According to phylogenetic analysis green and red algae are close to green plants. However, other algae and diatoms are not so much related and euglenoid algae appear even more distant. Below is given the account of different phyla of Algae.

Phylum: Chlorophyta (green algae)

Green algae contain chlorophylls a and b and carry cellulose in cell walls. It is believed that terrestrial plants have evolved from green algae. Green algae also store starch as plants do. The species from Chlorophyta are found in freshwater and soil, though few have marine habitat too. Most of green algae are microscopic. The type genus of green algae is *Chlamydomonas*.

Phylum: Charophyta (Stoneworts/Brittleworts)

The stoneworts make a dense covering on the bottom of shallow ponds, and precipitate calcium and magnesium carbonates forming a lime stone covering.

Phylum: Euglenophyta (Euglenoids)

Euglenoids also carry chlorophylls a and b though no cell wall is present. Most of the species are found in freshwater though few are of marine habitat too. Structure wise species are unicellular and flagellated. Euglenoids store paramylon as reserve food material. The type genus of Euglenoids is *Euglena*. Cells of *Euglena* can spontaneously lose chloroplasts and can exist as heterotrophic organisms. These show phylogenetic relationships to protozoan *Trypanosoma*.

Phylum: Pyrrhophyta (dinoflagellates)

Dinoflagellates are flagellated unicellular algae also called plankton. Many members of the dinoflagellates are free living though some of the species can live in symbiotic relationships with animals which make up reefs in marine habitats. The presence of cellulose in the membrane is responsible for the rigid structure of dinoflagellates. This algae carries pigments chlorophylls a and b and xanthophylls and reserve food in the form of starch. Species of dinoflagellates also produce neurotoxins and have been responsible for killing of millions of fishes in last couple of decades. Species of Genus *Alexandrium* produce neurotoxins which causes 'paralytic shellfish poisoning'. When large numbers of dinoflagellates are eaten by mollusks, toxins get concentrated inside mollusks and affect humans who eat them. *Alexandrium* occasionally grow as dense suspension and give ocean deep red color, leading to 'red tides'.

Phylum: Chrysophyta (Golden-brown algae, diatoms)

These are unicellular or filamentous algae having cell walls made of pectin and layer of silica. Characteristic pattern of cell wall helps in diatom identification. Siliceous contents (diatomaceous earth) in cell wall make them very resistant to decay and for this reason some of the oldest fossils belong to diatoms. Diatomaceous earth is used in detergents, fine abrasive polishes, paint removers, decolorizing and deodorizing oils and fertilizers. These genera are found in soil, freshwater as well as marine environment. Diatoms contain chlorophylls a and c, carry out photosynthesis and store energy in the form of lipids. Diatoms have also been associated with neurological disease through a toxin called 'domoic acid'. Example: *Cyclotella*

Phylum: Phaeophyta (Brown algae)

Brown algae can be filamentous to leafy and in some genera massive and plantlike. They carry pigments chlorophylls a and c and xanthophylls. Most brown algae have marine habitat and are found in coastal waters. The cell walls of algae are made up of cellulose and alginic acid. Algin, a thickener used in food industry and cosmetics is extracted from the cell walls of brown algae. One of the brown algae, *Laminaria japonica* is used in the gynecological procedures. Brown algae store energy in the form of carbohydrates (laminarin and mannitol).

Phylum: Rhodophyta (red algae)

Red algae live in deep marine habitats. These algae contain pigments such as chlorophylls a and d, phycocyanin and phycoerythrin. Presence of red pigments helps algae to absorb blue light that penetrates deep in ocean. The cell walls of red algae are made up of cellulose while starch is stored as carbon reserve. Agar used in microbiological media and food industry is derived from red algae. Example: *Corallina*

6. Viruses

Definition: Viruses are obligate intracellular parasites, are inert outside a living host and can even be crystallized. They can be regarded as exceptionally simple living microorganism in comparison to all other living organisms. By definition viruses are sub-microscopic entities that (i) contain a single type of nucleic acid (either DNA or RNA), (ii) multiply within a living cell and make use of macromolecule synthesizing machinery of the host cell, (iii) contain a protein coat (enclosed some times by an envelope containing lipids, proteins and carbohydrates) surrounding the nucleic acid. Viruses can infect bacteria, algae, fungi, protozoa, invertebrates, vertebrates and plants. Being obligate pathogens they are of serious medical, social and economical concerns. Viruses that infect bacteria are called bacteriophages or phages. Since their discovery, viruses have provided an easy system to look into the complications of molecular biology and solved a number of basic biological questions. Hershey and Chase by the use of bacteriophage as model organism provided evidence that the DNA, not protein, was the genetic material. Now a days viruses find a wide range of applications in biotechnology and research.

6.1 Viral structure and morphology

Nucleic acid: A virus can have either double stranded (ds) or single stranded (ss) DNA or RNA as the genetic material. The nucleic acid can be linear or circular depending on the virus and in some viruses the genome is segmented i. e. present in several separate segments (Influenza virus). The total amount of nucleic acid varies from a few thousand nucleotides (nt) to few hundred thousand nucleotides, for example, polio virus contains a ss RNA genome of about 7 kb (1kb = 1Kilobases = 1000 nucleotides) whereas pox virus contains a ds DNA genome of 250-350 kb.

Capsid (protein coat): A protein coat called, capsid surrounds the nucleic acid of a virus and protects it from nuclease enzymes present in biological fluids and helps in virus's attachment to the host cell. Capsid is composed of protein subunits called capsomeres. The arrangement of capsomeres is characteristic of a particular type of virus and based on capsid architect viruses are

divided into (i) helical viruses, (ii) icosahedral viruses and (iii) complex viruses. **Helical viruses** look like long rods that may be flexible or rigid and nucleic acid is present inside the hollow, cylindrical capsid as a helical structure. The capsomeres are arranged on the nucleic acid in helical manner. The first virus discovered was Tobacco Mosaic Virus (TMV) and it has a helical symmetry. Other important viruses having capsids with helical symmetry include those causing mumps, measles, influenza and Ebola haemorrhagic fever. **Icosahedral viruses** have circular or hexagonal appearance. Icosahedral capsid consists of capsomeres arranged in a regular array of 20 triangular faces and 12 corners or vertices. Nucleic acid is enclosed inside the icosahedral capsid. Viruses causing herpes, common cold, dengue fever and polio are examples of icosahedral viruses. Viruses that do not fall in any of the above categories are called **complex viruses**. For example bacteriophages of T series (T1, T2, T3 and so on) contain icosahedral capsid (head that encloses the nucleic acid) with additional structures like a tail (helical symmetry), tail sheath and tail fibers attached to it. Another example of complex virus is poxvirus, which lacks a clearly identifiable capsid.

Envelope: In some viruses, the capsid is surrounded by an envelope composed of lipids, proteins and carbohydrates and is derived from host cell plasma membrane, nuclear membrane or endoplasmic reticulum/golgi complex membranes depending on the site of replication and mode of release of the virus from the host cell. Envelopes may or may not be covered by spikes which are usually encoded by the viral genome and are glycoproteinaceous in nature. These spikes are involved in the receptor mediated attachment of the virus to the host cell. For example, Influenza virus has haemagglutinin spikes which confer the virus with the property to clump red blood cells and binding of the virus to its receptor on the host cell, similarly rabies virus has G glycoprotein spikes and SARS coronavirus has S protein spikes on its surface.

6.2 Classification of viruses

Viruses were initially classified according to the symptoms of diseases they caused, for example, the group of hepatitis viruses included all the viruses that caused similar symptoms of hepatitis but with increasing knowledge of the biochemical properties, nature of nucleic acid (whether DNA or RNA, single stranded or double stranded) and the site and mode of replication and with the discovery of more and more number of viruses that caused infections not only in plants and vertebrates but also in bacteria, archaeobacteria, fungi, algae, protozoa and invertebrates, an International Committee on the Taxonomy of Viruses (ICTV) was constituted in 1966. Viruses are grouped into families based on (a) type of nucleic acid, (b) mode of replication, and (c) morphology. The suffix –virus, -viridae and –ales are used for genus, family and order names, respectively. Viral species is a group of viruses sharing the same genetic information and host range and are designated by descriptive names such as human herpes virus (HHV), with subspecies designated by a number (HHV-1).

6.2.1 Viruses that multiply in humans and other vertebrates

6.2.1.1 Viruses with double stranded DNA genomes

Family: Adenoviridae

This family consists of viruses of non-enveloped icosahedral particles of 60-90 nm in length having a fiber protein at each vertex. Each viral particle contains monopartite ds DNA genome of

25-42 kb. Human adenovirus type 2 or type 5 causes respiratory infection in humans, while some members cause tumors in animals.

Family: *Papillomaviridae*

The members of *Papillomaviridae* family consist of non-enveloped icosahedral particles of 55nm diameter. Each viral particle contains one molecule of double stranded, covalently closed, circular DNA of 8 kb size. The members include human papilloma virus type 1-60 which mostly induce wart like tumors in humans whereas HPV-16 and HPV-18 are involved in more serious infections causing cervical cancer.

Family: *Polyomaviridae*

The members of this family are non-enveloped icosahedral particles of 40 nm diameter. Each viral particle contains one molecule of double stranded, covalently closed, circular DNA of 5 kb length. The members includes simian virus 40 (SV40), and murine polyoma virus. Most members are oncogenic.

Family: *Herpesviridae*

Members of *Herpesviridae* are enveloped particles of 120-220 nm diameter with icosahedral nucleocapsid. Each virus contains one linear molecule of ds DNA of 120-240 kb size. *Herpesviridae* is a very large family with some oncogenic viruses. Examples include important genera like *Simplexvirus* (HHV-1 and HHV-2) causes herpes simplex, *Varicellovirus* (HHV-3) causes chickenpox, herpes zoster, *Lymphocryptovirus* (HHV-4) causes Burkitt's lymphoma (cancer of blood cells in human) and HHV-8 causes Kaposi's sarcoma (with greatly increased incidences in AIDS patients).

Family: *Poxviridae*

Members of *Poxviridae* are enveloped, brick-shaped virions of 300 x 200 x200 nm dimension. Viruses have complex structure with one or two lateral bodies and a biconcave core and one linear molecule of dsDNA of 130-375 kb length. Example includes *Orthopoxvirus* (small pox and vaccinia viruses) and *Molluscipoxvirus* causing molluscum contagiosum (wart like skin lesion).

Family: *Hepadnaviridae*

Viruses of *Hepadnaviridae* are enveloped, 40-48 nm sized particles with icosahedral nucleocapsid. Each particle contains one circular partially double stranded DNA molecule of 3 kb size. These are 'reversiviruses' that replicates via RNA intermediate and reverse transcriptase. Example Includes *Hepadnavirus* (hepatitis B virus), which causes hepatitis and liver cancer in humans and ducks.

6.2.1.2 Viruses with single stranded DNA genomes

Family: *Parvoviridae*

Members of *Parvoviridae* are non-enveloped icosahedral particles of 18-26 nm dimensions. Each virus contains one linear molecule of ssDNA where replication occurs in the nucleus. Examples include canine and feline parvovirus and also *Erythrovirus* that causes 'fifth' disease of children.

6.2.1.3 Viruses with double stranded RNA genomes

Family: *Reoviridae*

Reoviridae is a large family with members which infect vertebrates, insects and plants. Typical viruses of *Reoviridae* are non-enveloped icosahedral particles of 60-80 nm length with 10-12 segments of linear dsRNA of 18-30 kb size. Replication in *Reoviridae* occurs in the cytoplasm. Genetic reassortments and recombinations are common in mixed infections evolving into new genetically stable hybrid viruses. Example: *Rotavirus* a common cause for infantile diarrhea.

6.2.1.4 Viruses with positive sense single stranded RNA genomes

Family: *Calciviridae*

Positive sense single stranded RNA genomes can directly act as mRNA. Members of *Calciviridae* are non-enveloped icosahedral particles with 35-40 nm length. Each particle contains one molecule of linear positive sense ss RNA of 7.5 kb. Examples of *Calciviridae* include human calcivirus, Norwalk virus and hepatitis E virus.

Family: *Picornaviridae*

Members of *Picornaviridae* are non-enveloped icosahedral 30 nm particles with one molecule of linear positive sense ssRNA of 7-8 kb size. Species of this family include many important pathogens of humans and other vertebrates. Examples include *Enterovirus* (at least 70 human Enteroviruses are known including polio, echo-, coxsackie-, human hepatitis A virus) and *Rhinovirus* (cause common cold).

Family: *Coronaviridae*

Viruses of *Coronaviridae* are enveloped, 120-160 nm size particles with club shaped protein spikes inserted into the lipid membrane. Each particle contains a helical nucleocapsid with one molecule of linear positive sense ss RNA of 28-31 kb. Replication of *Coronaviridae* viruses occurs in the cytoplasm, e.g., severe acute respiratory syndrome (SARS) virus, human coronavirus OC43, Bovine corona virus and avian infectious bronchitis virus. These viruses are usually associated with upper respiratory tract infections and common cold.

Family: *Flaviviridae*

These viruses have envelope, 40-60 nm length with an icosahedral nucleocapsid of 25-30 nm and one molecule of linear positive sense ssRNA of 9.5 - 12.5 kb size. Replication of viruses occurs

in the cytoplasm and the infection usually spreads by arthropod vectors. Examples: *Flavivirus* causing Yellow fever, Dengue, West Nile and Japanese encephalitis and *Hepacivirus* causing hepatitis C in humans.

Family: *Togaviridae*

Members of *Togaviridae* are enveloped 70 nm particles with an icosahedral nucleocapsid and one molecule of linear positive sense ssRNA of 9-12 kb size. Examples of *Togaviridae* include *Rubivirus* (causes Rubella), Sindbis virus and Semliki forest virus.

6.2.1.5 Viruses with negative-sense/ambisense single stranded RNA genomes and a virion associated RNA- dependent RNA polymerase

Family: *Arenaviridae*

Members of *Arenaviridae* are enveloped 100 nm particles with club shaped spikes and two helical nucleocapsids and two molecules of linear ambisense ss RNA of 7.5 and 3.5 kb, respectively. Viruses replicate in cytoplasm and are divided into two groups; Old World arenavirus (lassa virus) and New World arenavirus (Tacaribe, Junin and related viruses).

Family: *Bunyaviridae*

These are enveloped 100 nm particles with 10 nm spikes and three helical nucleocapsids each with one molecule of linear negative sense ss RNA (large RNA: 6 to 12 kb, medium RNA: 3.5 to 6 kb and small RNA: 1 to 2 kb). Examples of *Bunyaviridae* include *Bunyavirus* (California encephalitis virus) and *Hantavirus* (Hantaan hemorrhagic fever).

Family: *Filoviridae*

Members of *Filoviridae* are enveloped long filamentous particles of 800 x 80 nm dimension with helical nucleocapsid. Each particle contains one molecule of linear negative sense ssRNA of 9 kb. Examples of *Filoviridae* include Marburg, Ebola and Reston viruses. These are highly pathogenic for humans.

Family: *Orthomyxoviridae*

Members of *Orthomyxoviridae* are enveloped, pleomorphic (sometimes filamentous), 100 nm particles with a dense layer of haemagglutinin and neuraminidase protein spikes. Each virus contains six to eight helical nucleocapsids each enclosing one molecule of linear negative sense ss RNA of 12-15 kb size. Genetic reassortments and recombinations are common in mixed infections. Example includes *Influenzavirus* (influenza viruses type A, B and C).

Family: *Paramyxoviridae*

Members of *Paramyxoviridae* are enveloped, pleomorphic, 150-200 nm size particles with a dense layer of fusion protein and haemagglutinin (with or without neuraminidase activity) protein spikes. Each particle contains one helical nucleocapsid enclosing one molecule of linear

negative sense ss RNA of 13-16 kb size. Family includes *Paramyxovirus* (causes parainfluenza, mumps) and *Morbillivirus* (causes measles).

Family: *Rhabdoviridae*

Members of *Rhabdoviridae* are enveloped bullet shaped particles with helical nucleocapsid and one molecule of linear negative sense ss RNA of 11-15 kb size. Family includes viruses such as *Vesiculovirus* (vesicular stomatitis virus) and *Lyssavirus* (rabies virus).

6.2.1.6 Viruses with ss RNA genomes that replicates through a DNA intermediate

Family: *Retroviridae*

The members of *Retroviridae* are enveloped 80 - 100 nm sized particles with spikes. Viral nucleocapsid can be isometric or a truncated cone and contains two identical copies of linear positive sense ss RNA of 7-11 kb size. Each virion contains a reverse transcriptase that transcribes genomic RNA into DNA (which is called provirus) which gets integrated into the host DNA. Some members of *Retroviridae* are carcinogenic. Family includes important pathogens such as human immunodeficiency virus type 1 (HIV-1) and 2 (HIV-2), Human T-cell lymphotropic virus (HTLV) types 1-3, murine leukemia virus and Rous sarcoma virus.

6.2.2 Viruses that multiply in Bacteria

6.2.2.1 Viruses with double stranded DNA genomes

Families: *Siphoviridae*, *Myoviridae* and *Podoviridae*

Viruses that multiply in bacteria are called as bacteriophages. Members of families *Siphoviridae*, *Myoviridae* and *Podoviridae* are non-enveloped particles with complex symmetry with an icosahedral head of 60-80 nm and a helical tail of 20-450 nm. The particles contain one molecule of linear ds DNA. Examples include coliphage T1, T2, T4, T6, Lambda (λ), Chi (χ), phi (ϕ) 80, P1, P2, and Mu.

Families: *Tectiviridae* and *Corticoviridae*

Members of these families are non-enveloped and can form icosahedral particles of 60-63 nm. Each particle carries one molecule of linear or circular ds DNA. Examples include enterobacteria phage PRD 1 and *Pseudomonas* phage PM2.

6.2.2.2 Viruses with single stranded DNA genomes

Family: *Inoviridae*

The members of *Inoviridae* are non-enveloped rods which carry one molecule of ss DNA of 5-10 kb size. Length of the virion depends on the length of the DNA. Examples include Enterobacteria phages M13, f1 and fd.

Family: *Microviridae*

Microviridae members are non-enveloped icosahedral particles of about 23 nm. Each particle contains one molecule of circular ss DNA of 4.4 - 5.4 kb. Example includes Enterobacteria phage phiX174.

In addition to above families, there is one family each of viruses containing ds RNA as well as ss RNA genomes and are named *Cystoviridae* (*Pseudomonas* phage $\Phi 6$) and *Leviviridae* (enterophages MS2 and Q β), respectively.

6.2.3 Viruses that multiply in plants

6.2.3.1 Viruses with single stranded DNA genomes

Family: *Geminiviridae*

The members of this family have unique morphology with two incomplete icosahedra joined together. These are non-enveloped viruses and have nuclear replication and are usually transmitted in a persistent manner by whiteflies or leafhoppers. Family includes maize streak virus, beet curly top virus and bean golden mosaic virus.

6.2.3.2 Viruses with double stranded DNA genomes

Family: *Caulimoviridae*

Members of *Caulimoviridae* have non-enveloped icosahedral particles. The genomic DNA replicates via an RNA intermediate with the help of enzyme reverse transcriptase. The example includes cauliflower mosaic virus transmitted by aphids in a non-persistent manner.

6.2.3.3 Viruses with double stranded RNA genomes

Family: *Reoviridae*

Reoviridae is a large family with members infecting vertebrates, insects and plants. The members contain non-enveloped icosahedral particles of 60-80 nm length with 10-12 segments of linear ds RNA of 18-30 kb. Virus replicates in the cytoplasm and are transmitted through leafhopper or other insect vectors in a persistent manner. Genetic reassortments and recombinations are common in mixed infections. Family includes rice ragged stunt virus.

6.2.3.4 Viruses with positive sense single stranded RNA genomes

Families: *Comoviridae*, *Luteoviridae*, *Tombusviridae*, *Bromoviridae*

The viruses of these families are non-enveloped icosahedral particles of various sizes. Examples include important viruses such as cowpea mosaic virus (non-persistent in beetle vector), tobacco ringspot virus (nematode vector), barley yellow dwarf virus (semi-persistent in aphid vector), Tomato bushy stunt virus (transmitted mechanically) and brome mosaic virus (beetle vector).

Families: *Closteroviridae*, *Potyviridae*

The members of these families are non enveloped long flexible rods with helical symmetry. Examples include beet yellow virus, potato virus Y, Potato virus X and wheat streak mosaic virus.

6.2.3.5 Viruses with negative/ambi sense single stranded RNA genomes

Families: *Bunyaviridae* and *Rhabdoviridae*

Both these families have members that cause infection in animals also (characteristics discussed earlier). Examples for plant viruses include tomato spotted wilt virus and potato yellow dwarf virus respectively, and are transmitted by insect vectors in a propagative and persistent manner.

6.3 Transmission of plant viruses

Unlike animal viruses which have various routes of transmission including oral-fecal, blood borne, sexual, respiratory and vertical routes in addition to transmission through arthropod vectors, plant viruses are mostly transmitted through plant eating insect vectors and a few are transmitted mechanically through grafting and other procedures and a few are seed borne. Since the plant cells have cell wall which presents a barrier to the entry of virus, entry is almost always gained through the wounds. Insect vectors fulfill this requirement by damaging the plant tissues through their chewing or sucking mouthparts and inoculating the plant with the virus which they gain during feeding on an infected plant. All the types of plant insects are capable of transmitting viruses including thrips, aphids, leaf hoppers, grasshoppers, whiteflies, beetles, nematodes and even plant infecting fungi have been shown to be involved in transmission of plant viruses. Vectors transmit the viruses in three manners depending on the duration for which they keep on infecting new plants after feeding on an infected plant: persistent, non-persistent and semi-persistent. In **persistent transmission** insect shares a biological relationship with the virus (many a times the virus is able to multiply in the vector) and after feeding once on an infected plant, the insect keep on infecting new plants for a very long time, sometime through out its life span. A vector is said to be transmitting the virus in a **non-persistent** manner when it can transmit the virus mechanically only to one or a few plants. Here the virus does not share a biological relationship with the vector. **Semi-persistent** transmission is an intermediate form of transmission with a few characteristics each of persistent and non-persistent transmission.

7. Prions

Prions are infectious proteinaceous particles described for the first time in the year 1982 by Stanley Prusiner, while studying a neurological disease called scrapie in sheep. He observed that the infectivity of scrapie infected brain tissue reduced by treatment with proteases but not by treatment with radiation, suggesting the pure protein nature of the infectious agent. Nine neurological disorders have been recognized in animals that are caused by prions including mad cow disease in cattles, kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome and fatal familial insomnia in humans. These diseases run in families indicating a possible genetic cause but they are not purely inherited as mad cow disease originated from feeding scrapie-infected sheep meat to cattle and CJD has been transmitted with transplanted nerve tissues.

8. Viroids

Viroids are small, circular, infectious ss RNA (250-370 nucleotides long) without a protein coat. They cause diseases only in plants. Viroids do not encode any protein themselves but make use of host cell enzymes and proteins for their replication and are transmitted through seeds, vegetative propagation and vectors. Viroids include potato spindle tuber viroid, coconut cadang cadang viroid and avocado sunblotch viroid.

Suggested readings

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