MICROBIAL TAXONOMY

INTRODUCTION

- Diversity of living world is enormous.
- Scientists have discovered nearly 1.5 million species and still millions of species are unknown to us.
- To facilitate systematic study research and scientific communications ,the organisms are grouped into taxonomic categories.
- Aristotle (4BC), a greek Philosoper, was the first who made an attempt to classify living things.
- He classified entire living kingdom into two major groups Plants and animals.
- The modern basic of taxonomy was suggested by Carolus Linnaeus a Swedish naturalist.
- He is honored as **the Father of Taxonomy for his contribution on bionomial nomenclature system.**
- **Ernst Haeckel(1834-1914), a German scientist, introduced the Protista kingdom.**
- **Advances in the cell biology revised the system of classification.**
- **Edouard Chatton introduced the term prokaryote in 1937 for cell without nucleus.**
- **Robert Stanier defined prokaryotes as cell in which the nucleus material is not surrounded by a nuclear membrane.**

TERMINOLOGIES

1. **Taxonomy----- The science of classifying organisms that permits logical and informative system of naming and is useful for the identification of an organism.**
2. **Classification--- is the assignment of organism into an organized scheme of naming.**
3. **Taxa--- A group or category of related organism e.g Domain, Kingdom, Order, Class, Species or Genus**

Important characteristics of taxa are:

1. In general , member of lower level taxa are more similar to each other than the member of higher level taxa.
2. Members of specific taxa are more similar to each other.
   Microbial diversity known and estimated microbial species

Group	Known species	Estimated total species	Percentage
Bacteria	4760	40000	12
Fungi	69000	150000	5
Viruses	5000	130000	4
Algae	40000	60000	67
Protozoa	30800	100000	31

In 1969, Robert Whittakar of Cornell University , USA gave a system of classification of all living organisms that was based on the cellular organization and nutritional patterns known as Whittaker five-kingdom system of classification which is widely accepted system of classification amongst biologists . The five kingdoms are:

Kingdom Monera

• All monerans are unicellular **prokaryotes**: They generally lack organelles, have no true nuclei, and their DNA has little or no protein associated with it.

• The **cyanobacteria** are photosynthetic monerans of great ecological importance.

Kingdom Protista (slime molds protozoa and algae)

• unicellular **eukaryotic heterotrophic,some autotrophic**

Kingdom Fungi (Yeast, molds and mushrooms)

• The fungi include some unicellular and many multicellular organisms that obtain nutrients solely by absorption.

Kingdom Plantae

• Most plants live on land and contain chlorophyll in organelles called chloroplasts.

Kingdom Animalia

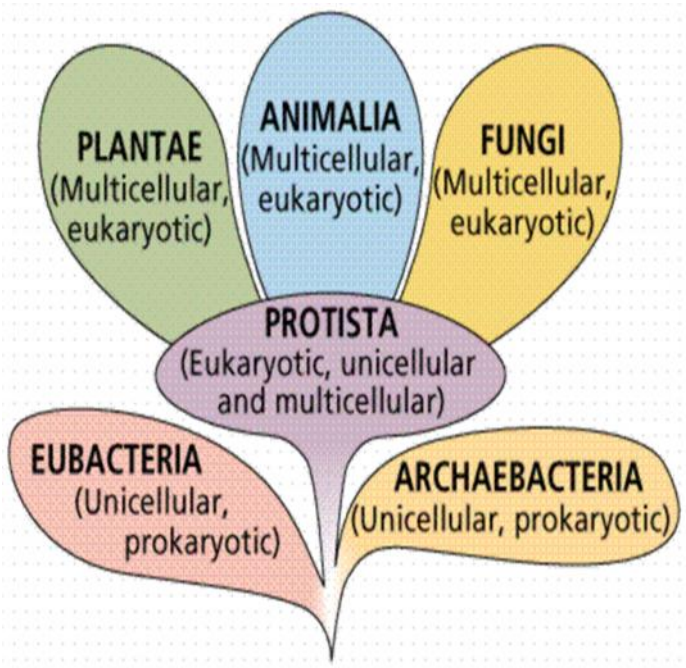
• All animals are derived from zygotes; most are macroscopic.

The Five-Kingdom System of Classification

	Monera (Prokaryotae)	**Protista**	Fungi	**Plantae**	**Animalia**
Cell type	Prokaryotic	Eukaryotic	Eukaryotic	Eukaryotic	Eukaryotic
Cell organization	Unicellular; occasionally grouped	Unicellular; occasionally multicellular	Unicellular; multicellular	Multicellular	Multicellular
Cell wall	Present in most	Present in most absent in others	Present	Present	Absent
Nutrition	Absorption, some photosynthetic, some chemosynthetic	Ingestion or Absorption some photosynthetic	Absorption	Absorptive, photosynthetic,	Ingestion; occasionally in some parasites by absorption
Reproduction	Asexual, usually by binary fission	Mostly asexual occasionally Both sexual and asexual	Both sexual and asexual often involving a complex life	Both sexual and asexual	Primarily sexual

Before we discuss the five-kingdom classification system and how it applies to microorganisms, we must emphasize that all living organisms, regardless of the kingdom to which they are assigned, display certain characteristics that define the unity of life. All organisms are composed of cells, and all carry out certain functions, such as obtaining nutrients and getting rid of wastes. The cell is the basic structural and functional unit of all living things. The fact that viruses are not cells is one reason they are not considered to be living organisms. All cells are bounded by a cell or plasma membrane, carry genetic information in DNA, and have ribosomes where proteins are made. All cells also contain the same kinds of organic compounds—proteins, lipids, nucleic acids, and carbohydrates. They also selectively transport material between their cytoplasm and their environment. Thus, although organisms may be classified in very diverse taxonomic groups, their cells have many similarities in structure and function.

No single classification system is completely accepted by all biologists. One of the most widely accepted is the **five-kingdom system** (Figure).



A major advantage of this system is the clarity with which it deals with microorganisms.

It places all **prokaryotes**, microorganisms that lack a cell nucleus, in the kingdom Monera (Prokaryotae). It places most unicellular **eukaryotes**, organisms whose cells contain a distinct nucleus, in the kingdom Protista. (Margulis proposed a very similar five kingdom system in 1982, but she referred to the kingdom of simple eukaryotes as Protoctista instead of Protista.)

The five-kingdom system also places fungi in the separate kingdom Fungi.

Kingdom Monera

The kingdom **Monera** (mo-ner_ah) is also called the kingdom **Prokaryotae**, as suggested by the French marine biologist Edouard Chatton in 1937. It consists of all prokaryotic organisms, including the eubacteria ("true bacteria"), the cyanobacteria, and the archaeobacteria (Figure 9.6).

All monerans are unicellular; they lack true nuclei and generally lack membrane-enclosed organelles. Their DNA has little or no protein associated with it. Reproduction in the kingdom Monera occurs mainly by binary fission.

The **cyanobacteria** (si_an-o-bak-ter_e-ah), formerly known as blue-green algae, are of special importance in the balance of nature. They are photosynthetic, typically unicellular organisms, although cells may sometimes be
connected to form threadlike filaments. Being autotrophs, cyanobacteria do not invade other organisms, so they pose no health threat to humans, except for toxins (poisons) some release into water.

Cyanobacteria grow in a great variety of habitats, including anaerobic ones, where they often serve as food sources for more complex heterotrophic organisms.Some “fix” atmospheric nitrogen, converting it to nitrogenous compounds that algae and other organisms can use. Certain cyanobacteria also thrive in nutrient-rich water and
are responsible for algal blooms—a thick layer of algae on the surface of water that prevents light from penetrating to the water below. Such blooms release toxic substances that can give the water an objectionable odor and even
harm fish and livestock that drink the water.

Archaeobacteria (ar_ke-o-bak_ter_e-ah) surviving today are primitive prokaryotes adapted to extreme environments. The methanogens reduce carbon-containing
compounds to the gas methane. The extreme halophiles live in excessively salty environments, and the thermoacidophiles live in hot acidic environments.

Kingdom Protista

Although the modern protist group is very diverse, it contains fewer kinds of organisms than when first defined by Haeckel. All organisms now classified in the kingdom
Protista are eukaryotic. Most are unicellular, but some are organized into colonies. Protists have a true membrane-enclosed nucleus and organelles within their
cytoplasm, as do other eukaryotes. Many protists live in fresh water, some live in seawater, and a few live in soil. Protists do not develop from an embryo, as plants and animals do, and they do not develop from distinctive spores, as fungi do. Yet, among the protists are the algae, which resemble plants; the protozoa, which resemble animals; The protists of greatest interest to health scientists are the protozoa that can cause disease.

Kingdom Fungi

The kingdom **Fungi** includes mostly multicellular and some unicellular organisms. Fungi obtain nutrients solely by absorption of organic matter from dead organisms.

Even when they invade living tissues, fungi typically kill cells and then absorb nutrients from them. Although the fungi have some characteristics in common with plants, their structures are much simpler in organization than true leaves or stems. Fungi form spores but do not form seeds. Many fungi pose no threat to other living things, but some attack plants and animals, even humans

Others such as yeast and mushrooms are important as foods or in food production.

Kingdom Plantae

The placement of most microscopic eukaryotes with the protists leaves only macroscopic green plants in the kingdom **Plantae**. Most plants live on land and contain chlorophyll in organelles called chloroplasts. Plants are of interest to microbiologists because some contain medicinal substances such as quinine, which has been used to treat microbial infections. Many microbiologists are very interested in plant-microbe interactions, particularly with regard to plant pathogens, which threaten food supplies.

Kingdom Animalia

The kingdom **Animalia** includes all animals derived from zygotes (a cell formed by the union of two gametes, such as an egg and a sperm). Although nearly all members of

this kingdom are macroscopic and therefore of no concern to microbiologists, several groups of animals live in or on other organisms, and some serve as carriers of microorganisms

Certain *helminths* (worms) are parasitic in humans and other animals. Helminths include flukes, tapeworms, and roundworms, which live inside the body of their host. They

also include leeches, which live on the surface of their hosts.

Microbiologists often need to identify both microscopic and macroscopic forms of helminths

THREE DOMAIN CLASSIFICATION SYSTEM

In the 1978 Carl R.Woese, proposed another taxon larger than a kingdom. He called it a domain. The domain is a taxonomic category that is above the level of kingdom.

Domain consisit of three members Eukaryotes (Domain Eukarya), Eubacteria (Domain bacteria and Archaebacteria (**Domain Archaea)**

All the organism are placed in one of the three domains based on their nucleic acid studies.

They include: **Bacteria**, **Archaea**, and **Eukarya**.

Domain Bacteria

• All Bacteria are unicellular prokaryotes and include the eubacteria ("true bacteria").

Domain Archaea

• All Archaea are unicellular prokaryotes, having a cell wall made of materials other than peptidoglycan.

Domain Eukarya

• All are eukaryotic cells, having a true nucleus.

The Eukarya are further divided into 4 kingdoms:

Protista, Fungi, Animalia, and Planatae

Species	: Fundamental rank in classification/collection of strain
Genus	: clubbing together of two or more species.
Family	: collection of genus
Order	: collection of families
Class	: arranging together of order
Phylum	: grouping together of class
Kingdom	: collection two or more phylum

What is Microbial Taxonomy?

Classification, nomenclature and identification comparise taxonomy of microorganisms. On the basis of common characters or properties, a set of organism is considered into groups (taxa).

There are no formal rules to define the taxa. On the other hand ,nomenclature is the name given for defined taxa as also governed by bacteriological code of nomenclature.

The features that are used to differentiate various organism often have to do with the fundamental basis for arranging the organism into taxonomic groups.

Difference between identification and classification

Identification	**classification**
1. It might be based on a phenotypic Character that is group to correlated well With generic information	It might be based on a DNA/DNA hybridization study
2. The character chosen for an identification Scheme should be easily determined.	Quite difficult to determined
3. In identification character should also Be few in number	large number of characters

1. CRITERIA OF CLASSIFICATION

These criteria sometime do not help in characterization of these genera or species which are not characterized by traditional biochemical or physiological test.

Serological test have limited role in classification but have importance in the identification. It is important to note that identification may not be based on only a few test, but rather on the whole battery of test .

Genetic tools are the modern one for identification of bacteria, based on the detection of as specific portion of an organisms genetic material.

2. GENETRAL METHOD OF C LASSIFYING BACTERIA

i) The Intuitive Method

A large array of microbiologist study the characterstics of organisms(morphology, physiological, biochemical , genetical ,molecular), sometimes it is difficult to assign an organism based on all the characters because a character may be important to a particular microbiologist may not be important to another.

ii) Identification

Identification is an important and practical aspect of taxonomy

It is most significant for clinical microbiologist for proper diagnosis of a disease.

Several characteristics are commonly used for the identification of bacteria and are as follows:

Morphological characteristics

Study of external appearance of organism is called as morphology

It includes the size, shape, arrangement and detection of endospore, pilli, capsule, and flagella.

Morphological studies consist of Grams staining, acid fast staining, spore staining, motility etc.

Biochemical characteristics

Microorganism are diverged with respect to metabolic pathways.

Study of end products of metabolism is of great significance in the identification of a microbe.

Following list of biochemical test widely used in routine identification

Sugar fermentation

- Organism is inoculated into medium containing a specific sugar. The growth of bacteria and end products(acid) of fermentation including gases are detected by specific system.
- **Starch hydrolysis**
- Organism is inoculated onto medium containing starch, after incubation the plate is flood with iodine solution, clear zone around colonies indicate the presence of starch degrading enzyme,

- **Catalase**
- Hydrogen peroxide is added over the growth of an organism on the agar plate, release of oxygen gas bubbles indicate the presence of catalase that oxidizes H2O2 to H2O and O2
- **Oxidase,**
- Bacterial culture place on oxidase disc containing tetramethyl paraphenylene diamine dil HCL colour change indicate positive test.
- **Citrate utilization,**
- Organism is inoculated into citrate agar medium in which citrate is act as carbon source. the presence of growth in medium test is positive.
- **Indol,**
- Organism is inoculated into peptone medium containing tryptophan.It is detected by using Kovac's reagent.
- **Methyl *red***
- Organism is inoculated into MR-VP medium after incubation methyl red indicator is added, presence of acid cause an indicator colour change (red)
- **Urease**.
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- Production of ammonia is detected by phenol red indicator. Pink red colour development is positive test.

Culture characteristics

Culture is an active growth of microbe in a suitable medium.

- The macroscopic appearance on different kinds of media is an important characteristics for the identification
- **Nutrient agar slant :**
- Nutrient agar is prepared by allowing the nutrient agar to solidify in slanting position within test tube. The appearance of different types of growth is important for identification of microorganism.
- Filiform :uniform growth along the line of inoculation
- Echinulate :margines of growth exhibit toothed appearance
- Beaded : Separated colonies along the line of inoculation.

  Rhizoid :Root like appearance

- **Nutrient broth**: Diffused surface and sediment type of growth in nutrient broth gives idea about oxygen demand of bacteria. The surface growth pattern is an important characteristics for the identification.
- Ring : Growth on surface associated with wall
- Pellicle : Thick pd like growth on surface
- Flocculent : Tuft of wool
- Membranous : sheet like
- Sediment : Growth is at bottom.

- **Nutrient agar plate:**
- The colony characteristics are most frequently used as growth characteristics for identification of bacteria
- Size : colony can be measured in millimeter
- Shape : colony is the specific feature of a microbe
- Colour : Several bacteria produce pigmentation pattern
- Opacity : degree of opacity may exist in opaque, transparent and translucent.

- Margine : outer edge of colony

Physiology

Microorganism grow at optimum physiological condition.

- **Growth temperature**
- **Psychrophilic**
- **Mesophilic**
- **Thermophilic**
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- **pH**
- **Acidophiles**
- **Neutrophiles**
- **Alkalophiles**
- **Osmotic tolerance**
- **Hypotonic**
- **Isotonic**
- **Hypertonic**
- **Oxygen requirement**
- **Aerobic**
- **Anaerobic**
- **facultative**
-

Serology

Which deal with blood serum and immune response.

- A branch of science, which deals with blood serum and immune response is called as serology. Microbes are antigenic. Antigens are any foreign substance that provoke antibody production by the host.
- Antigen antibody reaction is highly specific.
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- **Phage typing:**
- Bacteiophages or phages are viruses that infect bacteria. Phages typing differentiates microbial types by using phages.

BINOMIAL NOMENCLATURE

- It is more convenient, if a specific name is assigned to an organism rather than providing its elaborate description.
- Carl Linnaeus a Swedish naturalist, developed the system of binomial nomenclature.
- Every identified living organism has been assigned a binomial name, except viruses, which do not have binomial name.

Following are some of important point to remember regarding binomial nomenclature:

1. If an organism are known by different name in different countries, the confusion would result; binomial nomenclature thus avoid this confusion.
2. A scientific name of an organism consist of **two names** hence called as Binomial nomenclature. A first name is of the genus while a second name is of species i.e Genus come before species e.g *Escherichia coli*
3. The language generally used in the classification is Latin but rarely Greek terms are used. Advantage of using these language is that both language are dead languages.
4. When writing a scientific name the first letter of the genus name is always CAPITALIZED as it is always a noun e. g *Escherichia.* The species name is entirely in lower case letter and it is usually an adjective e.g coli.
5. The name of genera and species are usually printed in italics. In typewritten and handwritten manuscripts such name should be underlined.
6. The genus name may be used alone, but not the species name.

Molecular Taxonomy

- **Genetic relatedness**
- **A revolution in identification and classification has been brought about by examing the genetic material**
- **Mole % G + C content :**
- The **% G + C in DNA can be considered as one of the trait of comparison as it is a trait which does not change rapidly.**
- Bacterias who are difficult will show difference in G+C % and vice versa. DNA contains four nitrogenous bases : adenine (A), guanine (G), cytosine (C), and thymine (T). In double stranded DNA, A always pairs with T by two hydrogen bonds and G with C by three hydrogen bonds. The % G + C is preserved by each organism during evolution. In bacteria G + C contents are significantly variable, ranging between 25 and 75 %. The variation in same species is less than 2.5 %. Identical G + C value does not prove taxonomic identity. However differences in G + C could greatly differ in primary structure of chromosomal DNA.
- **Mole%G + C Content =(G + C)X100/(A+T+G+C)**
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- **G+C contents of some microorganism are as follows**
- Microorganisms %G+C
- Bacteria
- Bacillus 32-62
- Clostridium 21-54
- Escherichia 48-52
- Mycobacterium 62-70
- Algae
- Spirogyra 39
- Fungi
- Candida albicans 33-35
- Aspergillus niger 52
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-

- **It is measured by following different methods :**
- 1. Determination of the DNA density in cesium chloride density gradient by isopycnic ultracentrifugation. Under proper conditions, DNA density is proportional to G + C content. It does not give precise results.
- 2. Determination of melting temperature (Tm).

- **Ribotyping:**
- The coding sequences for rRNA are mostly conserved in the course of evolution.
- The 16S rRNA sequencing is primarily used, as it is more stable than 5S rRNA and easier to sequence than 23S rRNA.
- In oligonucleotide cataloging method, 16S rRNA is obtained after hydrolysis by Ti nucleases (isolated from *Aspergillus oryzae).*
- The Tl digested extract is then subjected for PAGE to separate the 16S rRNA.
- For identification a primer that is complementary to conserved sequences of the 16S rRNA is used.
- Recently, sequencing methods are followed after polymeraae chain reaction (PCR) for the amplification of 16S rRNA sequence.
- This technique is of importance for identifying the taxonomic position of non-cultivable microorganisms.

In addition to introducing the binomial system of nomenclature, Linnaeus also established a hierarchy of
taxonomic ranks: species, genus, family, order, class, phylum or division, and kingdom. At the highest level, Linnaeus divided all living things into two *kingdoms*—plant and animal. In his taxonomic hierarchy, much of which is still used today, each organism is assigned a species name, and species of very similar organisms are grouped into a genus. As we proceed up the hierarchy, several similar genera are grouped to form a *family*, several families to form an *order*, and so on to the top of the hierarchy. Some hierarchies today have additional levels, such as *subphyla*. Also, it has become accepted practice to refer to the first categories within the animal kingdom as *phyla* and to those within other kingdoms (we now have five) as *divisions*.
Recently, the five kingdoms have been grouped together into three *domains*, a new category even higher than kingdom. Domains will be discussed later in the chapter.The classifications of a human, a dog, a wolf, and a
bacterium are shown in Figure 9.2.

R. H.Whittaker felt that endosymbiosis could not account for all the differences between prokaryotes and eukaryotes.
He also felt that a taxonomic system should give more consideration to the methods organisms use to obtain

nourishment.Autotrophic nutrition by photosynthesis and heterotrophic nutrition by the ingestion of substances
from other organisms had been considered in earlier taxonomies. Absorption as a sole means of acquiring
nutrients had been overlooked. To Whittaker, fungi, which acquire nutrients solely by absorption, were sufficiently
different from plants to justify placing them in a different kingdom. Also, fungi have certain reproductive
processes not shared with any other organisms. Consequently, Whittaker proposed a taxonomic system in 1969
that separated the Protoctista into two kingdoms—Protista (pro-tis_tah) and Fungi—but retained the Monera,

T H E F I V E - K I N G D O M
C L A S S I F I C AT I O N SY ST E M

Before we discuss the five-kingdom classification system and how it applies to microorganisms, we must emphasize
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that define the unity of life.All organisms are composed of cells, and all carry out certain functions, such as obtaining nutrients and getting rid of wastes. The cell is the basicstructural and functional unit of all living things. The fact that viruses are not cells is one reason they are not considered to be living organisms. All cells are bounded by a cell or plasma membrane, carry genetic information in DNA, and have ribosomes where proteins are made.All cells also contain the same kinds of organic compounds—proteins, lipids, nucleic acids, and carbohydrates. They also selectively transport material between their cytoplasm and their environment.Thus, although organisms may be classified in very diverse taxonomic groups, their cells have many similarities in structure and function.

No single classification system is completely accepted by all biologists. One of the most widely accepted is the
five-kingdom system (Figure 9.5).A major advantage of this system is the clarity with which it deals with microorganisms. It places all **prokaryotes**, microorganisms that lack a cell nucleus, in the kingdom Monera (Prokaryotae) (_Chapter 4, p. 77). It places most unicellular **eukaryotes**, organisms whose cells contain a distinct nucleus, in the kingdom Protista. (Margulis proposed a very similar fivekingdom system in 1982, but she referred to the kingdom of simple eukaryotes as Protoctista instead of Protista.)
The five-kingdom system also places fungi in the separate kingdom Fungi.
The properties and members of each of the five kingdoms
are described below and summarized in Table 9.2.
A more detailed classification of bacteria is provided in
Appendix B.

Kingdom Monera

In 1998, Woese discussed theories about how the three domains may have arisen (Figure 9.12).The standard
view was that a universal common ancestor first split into **Bacteria** and **Archaea**, and then the **Eukarya** branched off from Archaea. A second view held that all three domainsarose simultaneously from a pool of common ancestors
that were all able to exchange genes with one another—hence, the universal genetic code.A third view sought to
explain how so many genes are present in Eukarya but lacking in Archaea and Bacteria.

The three domains Woese proposed are shown in Figure 9.13.The domain Eukarya contains all those kingdoms
of eukaryotic organisms—the animals, plants, fungi, and protists. The traditional kingdom Monera has been divided
into two domains: the domain Bacteria and the domain Archaea.A comparison of the three domains is presented
in Table 9.3.