Microbiology

US03CMIC 21

Elements of Microbiology - I

Unit- 4

Characterization, Classification and Identification of Microorganisms:

- Place of microorganisms in living world
- Whittaker's five kingdom concept
- Bergey's Manual of Systematic Bacteriology
- Major characteristics of Microorganisms
- Microbial Classification: Taxonomic groups
- General Methods of Classifying Bacteria -
  (a) Intuitive method (b) Numerical Taxonomy
  (c)Genetic Relatedness
- Nomenclature and Identification

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Characterization, Classification and Identification of Microorganisms: (Microbiology - Pelczar, Chan, & Krieg , 5th edition)

-Place of microorganisms in living world
[8 – 10 along with Table 1.1]
- Whittaker's five kingdom concept [11 and figure 1.6]
- Bergey's Manual of Systematic Bacteriology [12]
- Major characteristics of Microorganisms. [38 – 41]
-Microbial Classification: Taxonomic groups [41 – 42]
-General Methods of Classifying Bacteria: Intuitive method, Numerical Taxonomy , Genetic Relatedness
[42 – 45 upto few laboratories]
-Nomenclature and Identification
[45 – 47]{ figure 3.3 is not to be included }

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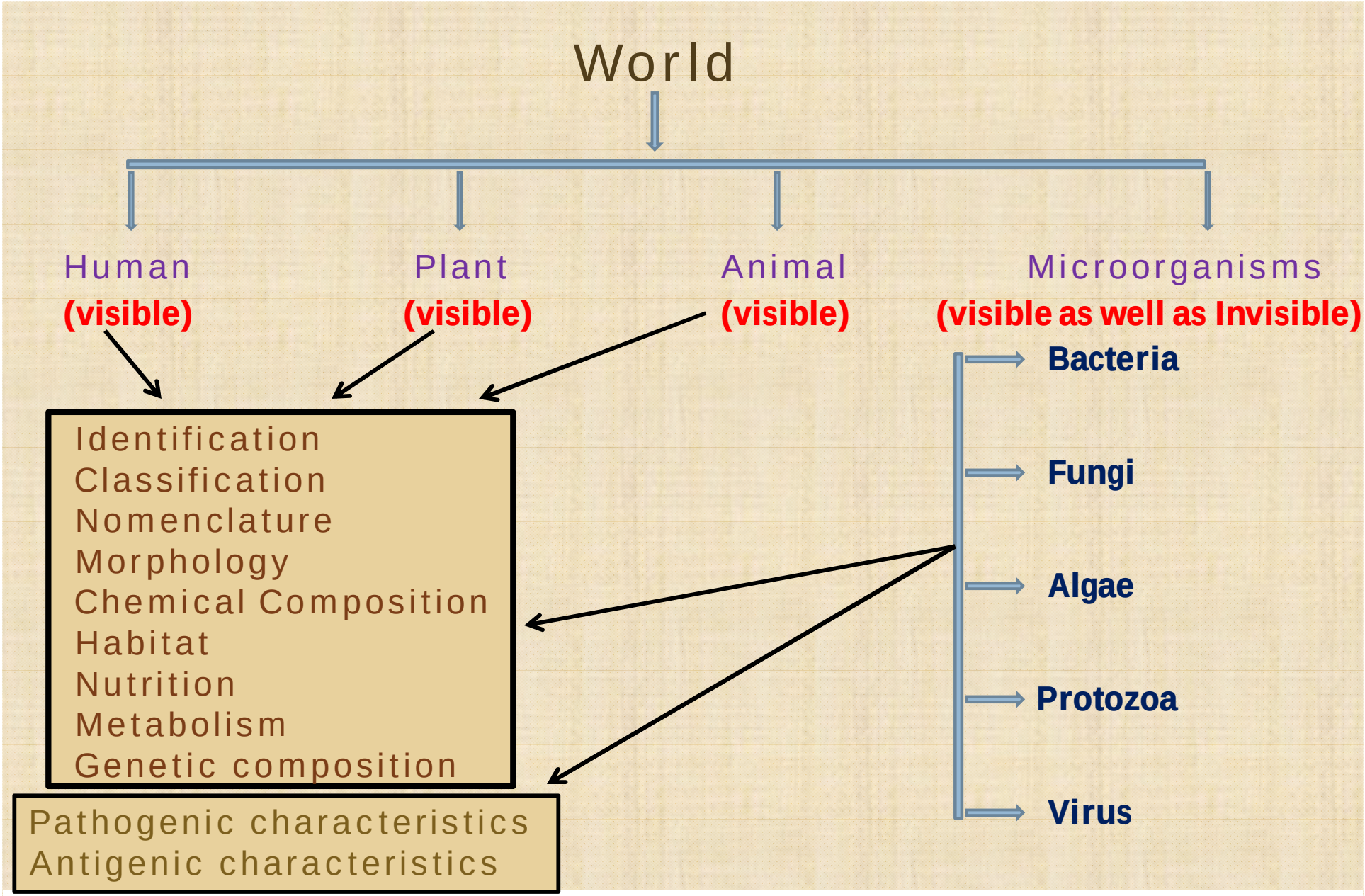

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Place of microorganisms in living world

- **Classification means the orderly arrangement of units under study into groups of larger units.**

- **Present day classification in biology was established by the work of Carolus Linnaeus (1770-1778), a Swedish botanist. His books on the classification of plants and animals are considered to be beginning of modern botanical and zoological nomenclature, a system of naming plants and animals.**

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- **Until 18th century the classification of living organisms placed all organisms into one of two kingdoms, plants and animal.**

- **But in microbiology we study some organisms that are predominantly plantlike ,and others that are animallike,and some share characteristics common to both plants and animals.**

- **That means there are organisms that do not fall naturally into either the plant or the animal kingdom, It was proposed that new kingdoms be established to include those organisms which typically are neither plants nor animals.**

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Haeckel's Kingdom

- One of the earliest of these proposals was made in 1866 by German zoologist, E.H. Haeckel.
- He suggested that a third kingdom ,Protista, which is formed to include those unicellular microorganisms that are typically neither plants nor animals.
- These organisms ,the protists, Included bacteria ,algae, fungi and protozoa.
  (Viruses are not cellular organisms and therefore are not classified as protists.)

- Bacteria are referred to as lower protists.
- Others- algae, fungi and protozoa are called higher protists.

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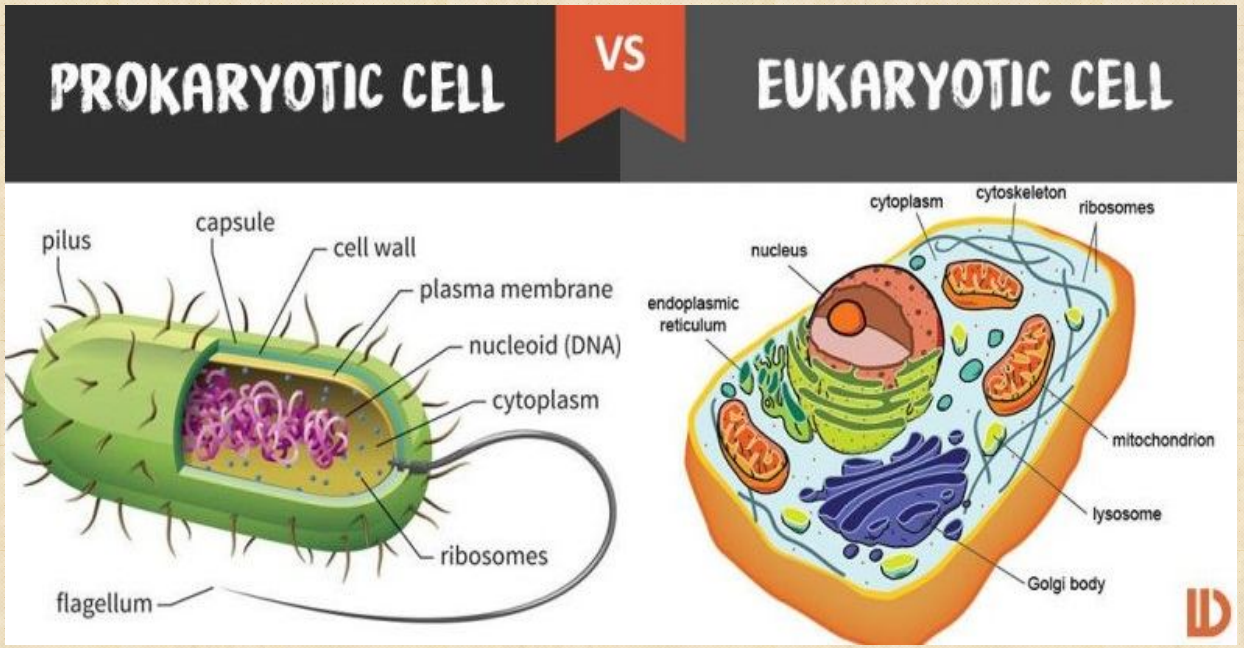
Prokaryotic and Eukaryotic Protists

Eukaryotic cells contain membrane-bound organelles, including a nucleus. Eukaryotes can be single-celled or multi-celled, such as you, me, plants, fungi, algae and insects. Prokaryotic cells do not contain a nucleus or any other membrane-bound organelle. Bacteria are an example of prokaryotes.



Viruses are left out of this classification scheme as they are parasite.

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Eukaryotic vs. Prokaryotic Cells

Characteristics	Eukaryotic Cells	Prokaryotic Cells
Definition	Any cell that contains a clearly defined nucleus and membrane bound organelles	Any unicellular organism that does not contain a membrane bound nucleus or organelles
Examples	Animal, plant, fungi, and protist cells	Bacteria and Archaea
Nucleus	Present (membrane bound)	Absent (nucleoid region)
Cell Size	Large (10-100 micrometers)	Small (less than a micrometer to 5 micrometers)
DNA Replication	Highly regulated with selective origins and sequences	Replicates entire genome at once
Organism Type	Usually multicellular	Unicellular
Chromosomes	More than one	One long single loop of DNA and plasmids
Ribosomes	Large	Small
Growth Rate/Generation Time	Slower	Faster
Organelles	Present	Absent
Ability to Store Hereditary Information	All eukaryotes have this ability	All prokaryotes have this ability
Cell Wall	Simple: Present in plants and fungi	Complex: Present in all prokaryotes
Plasma Membrane	Present	Present
Cytoplasm	Present	Present

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Whittaker's Five-kingdom Concept

- More recent and comprehensive system of classification.
- It was proposed by R.H.Whittaker in 1969.
- It is based on three levels of cellular organization:
  (1) **Prokaryotic**
  (2) **Unicellular eukaryotic**
  (3) **Multicellular eukaryotic**

- These three cellular organization which evolved to accommodate three principle mode of nutrition:
  (1) **Photosynthesis**
  (2) **Absorption**
  (3) **Ingestion**

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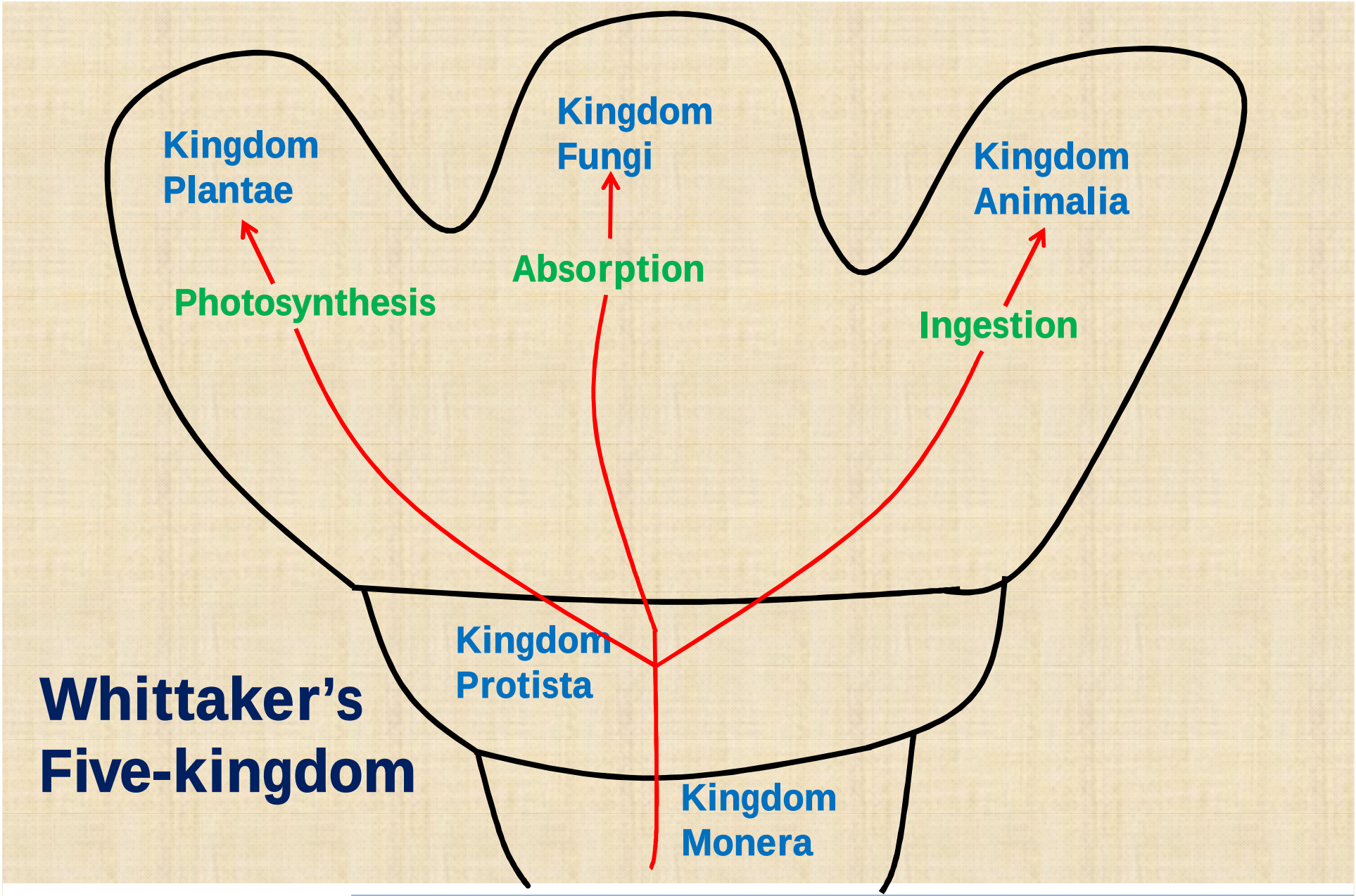

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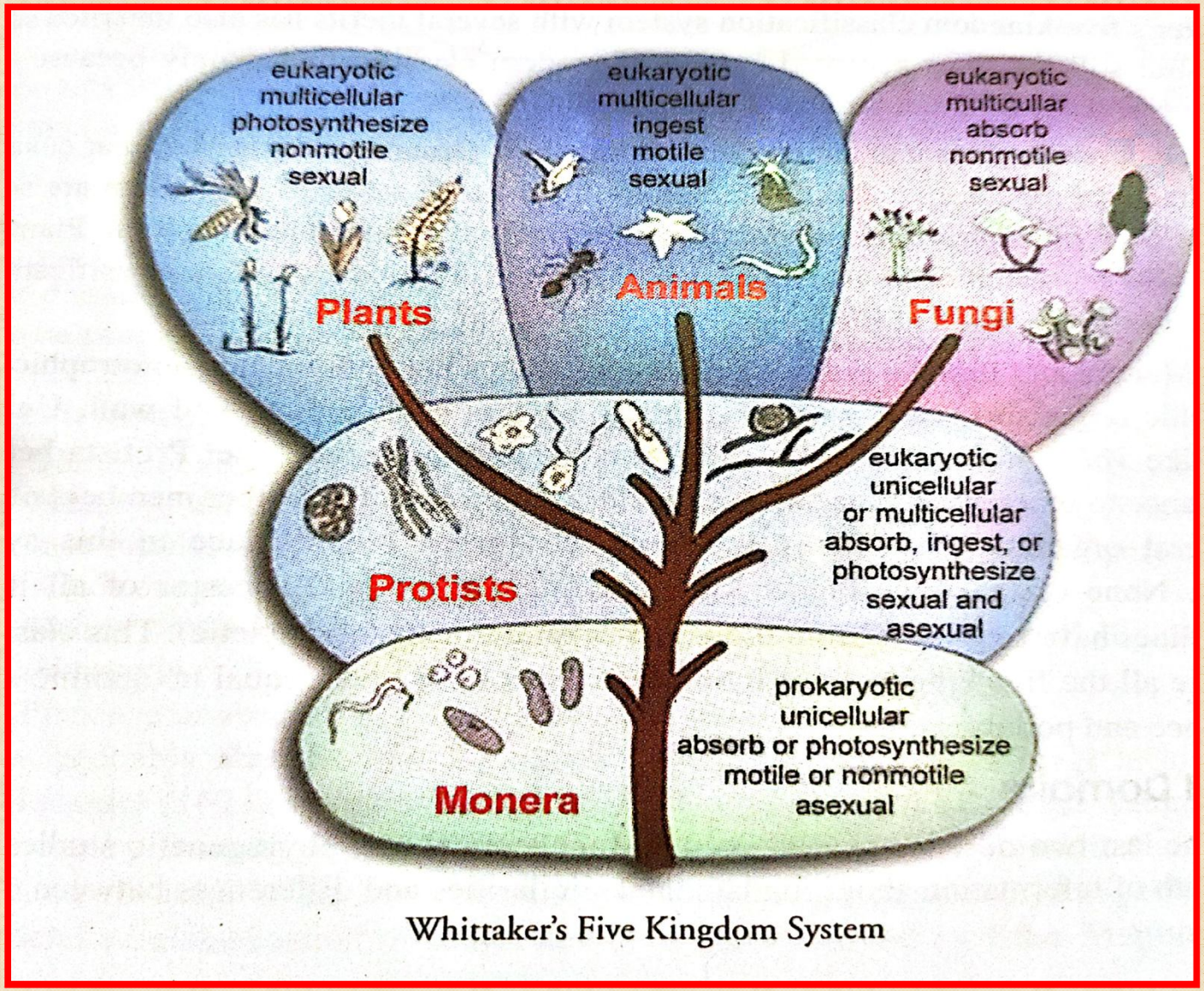

Whittaker's Five Kingdom System

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(1) Kingdom Plantae: (Mode of nutrition is photosynthesis)

Characteristics

- It includes eukaryotic multicellular autotrophs
- Plants are autotrophic in nutritional mode, making their own food by photosynthesis.
- They have multicellular sex organs and form embryos during their life cycles.
- Examples: Mosses, ferns and flowering plants are included in this kingdom.

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(2) Kingdom Fungi: (Mode of nutrition is absorption)

Characteristics

- It includes eukaryotic multicellular heterotrophs which are absorptive in their nutritional mode e.g. mushrooms.
- Most fungi are decomposers.
- They live on organic material, secrete digestive enzymes and absorb small organic molecules formed by the digestion by enzymes.

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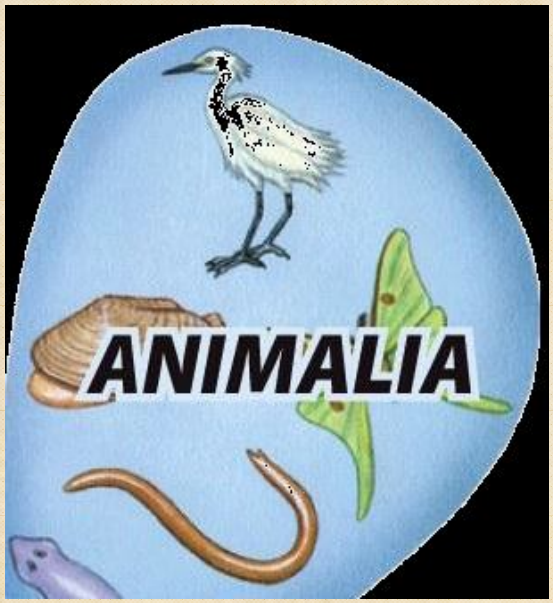
(3) Kingdom Animalia: (Mode of nutrition is Ingestion)



Characteristics

- It includes eukaryotic multicellular consumers.
- Animals live mostly by ingesting food and digesting it within specialized cavities.
- They lack cell wall and show movements

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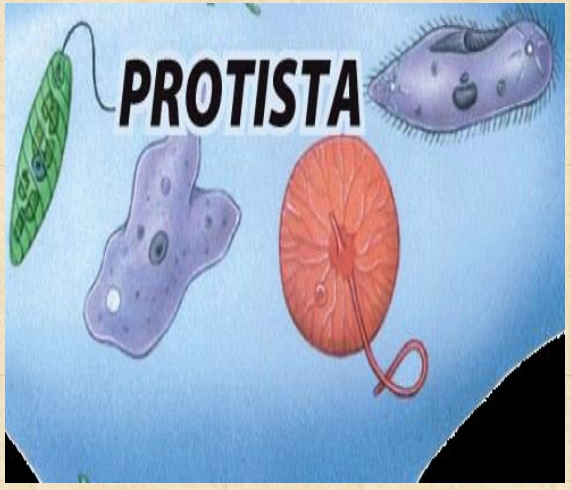
(4) Kingdom Protista:(Mode of nutrition is all three)



Characteristics

- It includes eukaryotic unicellular and simple multicellular organisms
- All three mode of nutrition represented here i.e.
  - Mode of nutrition of microalgae is Photosynthetic.
  - Mode of nutrition of protozoa is ingestive.
  - Mode of nutrition in some other protists is absorptive.

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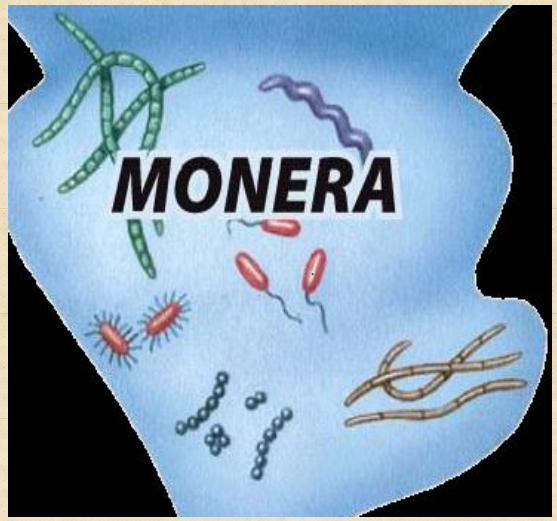
(5) Kingdom Monera

Characteristics

- All prokaryotes included in kingdom monera.
- They lack ingestive mode of nutrition.
- Monerans are unicellular
- Most are heterotrophic but some perform photosynthesis
- two different kinds of organisms i.e. bacteria and cyanobacteria
- There are two subkingdoms of Kingdom Monera:



(a) Archaebacteria-can live in the most extreme of environments

(b)Eubacteria -Is also called the true bacteria

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Bergey's Manual of Systematic Bacteriology

Bergey's a Manual of Systematic Bacteriology places all bacteria in the Kingdom Prokaryotes which in turn is divided into 4 divisions as follow:

Division 1 Gracilicutes
Prokaryotes with a complex cell-wall structure characteristics of Gram – negative bacteria

Division 2 Firmicutes
Prokaryotes with a cell-wall structure characteristics of Gram –positive bacteria

Division 3 Tennericutes
Prokaryotes that lack a cell-wall

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Division 4 Mendosicutes

Prokaryotes that show evidence of an earlier phylogenetic origin than those bacteria included in Divisions 1 and 2

- **Bergey's Manual is the international standard for bacterial taxonomy.**
- **A compatible manual of classification does not exit for fungi and algae or protozoa. However, there are schemes of classification for each group is available and for viruses is in the process of development.**

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Major characteristics of Microorganisms

Basic Terms:

- **Culture: A population of microorganisms.**

- **Pure Culture: A culture that consists of a single kind of microorganisms in an environment free of other living organisms.**
- **Colony: Distinct, compact masses of cells that are macroscopically visible.(A progeny of single cell)**

- **Culture medium: A mixture of nutrients used in the laboratory to support growth and multiplication of microorganisms.**

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- **In order to identify and classify microorganisms we must first learnt their characteristics.**

- **It is usually not feasible to study the characteristics of a single microorganism,. Because of its small size ; therefore we study the characteristics of a culture -a population of microorganisms.**

- **If we study the characteristics of a culture containing many microorganisms (usually millions or billions of cells of only one kind)which is like we are studying characteristics of a single organism.**

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Major characteristics of microorganisms

1. **Morphological characteristics**
2. **Chemical characteristics**
3. **Cultural characteristics**
4. **Metabolic characteristics**
5. **Genetic characteristics**
6. **Pathogenic characteristics**
7. **Ecological characteristics**
8. **Antigenic characteristics**

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1. Morphological characteristics

- Cell shape, size and structure; cell arrangement; occurrences of special structures and developmental forms; staining reactions and motility and flagellar arrangement.
- Requires pure culture for study.
- Microorganisms are very small and their size is usually expressed in micrometer μm and 1 μm is equivalent to 0.001 mm. So, different types of microscopy can be employed for such characterization.
- Routine microscopic examination requires the use of High power microscope which has magnification about 1000 diameter.
- Electron microscopy can help to see fine details of cell structure.

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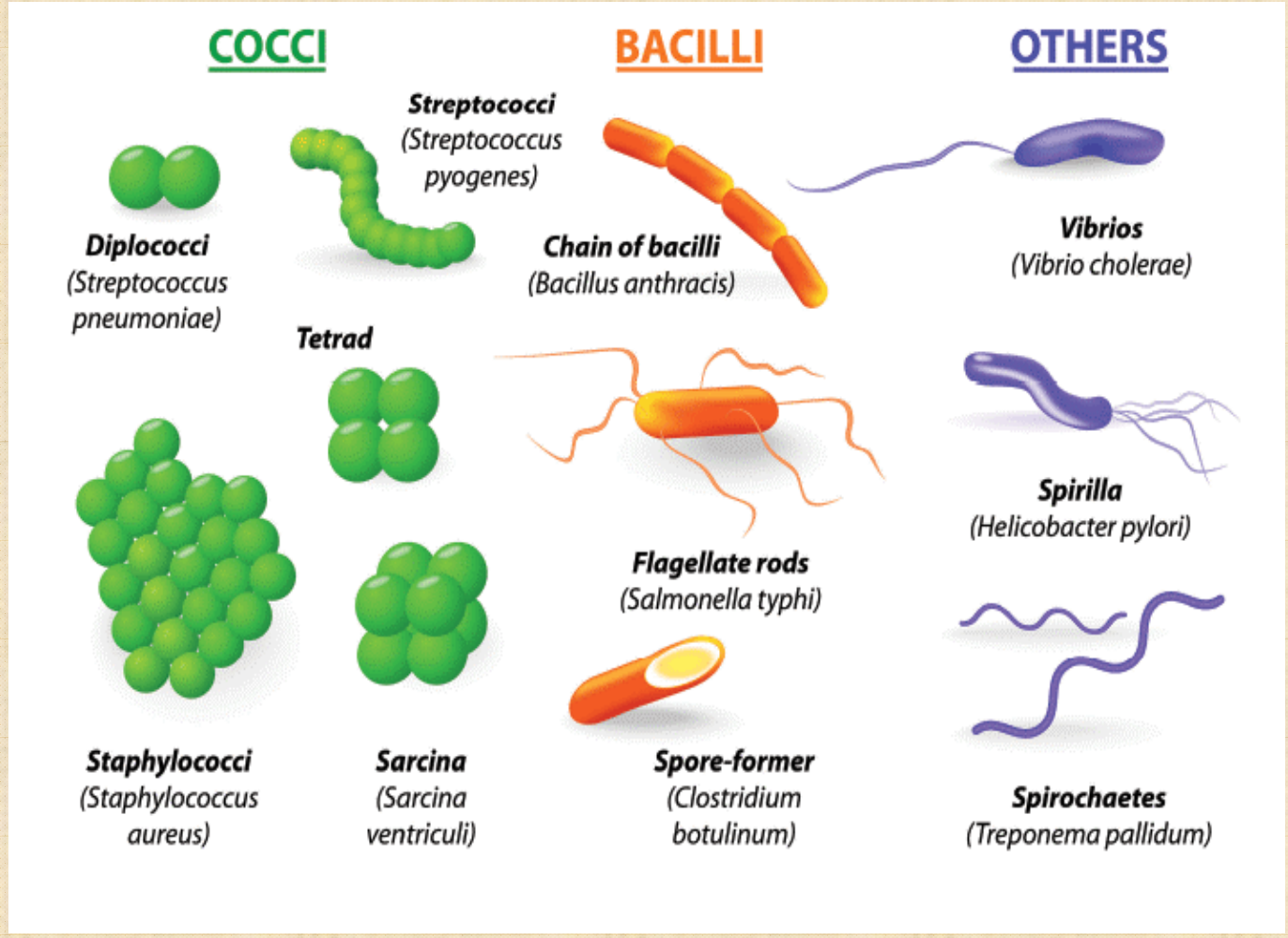

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2. Chemical characteristics

- **The various constituents of microbial cells contain a wide variety of organic compounds.**
- **Microorganisms have characteristic chemical composition with both quantitative and qualitative differences.**
- **Eg- (1) Occurrence of lipopolysaccharide in cell wall of gram negative bacteria; on other hand many gram positive have cell-walls that contain teichoic acid. Fungal and algal cell-walls are very different in composition from those of bacteria.**
  (2) Basic distinction among viruses is made depending on the basis of the kind of nucleic acid they posses, namely, DNA or RNA.

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3. Cultural characteristics

- Nutritional requirements and physical conditions required for growth and the manner in which growth occurs.
- Different types of culture medium: Culture containing inorganic compounds or organic compounds (amino acids, sugar, vitamins etc). Complex substances like peptone, blood cells or serum) may also be needed.
- Some microorganisms require living host cells for growth. E.g.- ricketssias need a culture of mammalian tissues to grow.
- Incubation temperature is also very important. Some bacteria require more than 40 °C while others grow below 20 °C. Human pathogenic bacteria grow at body temperature, 37 °C.

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- The gaseous atmosphere required for growth is also important: e.g. some bacteria requires oxygen for growth; oxygen is lethal to others and they can grow only in its absence.
- light also play important roles. e.g.- cyanobacteria uses light as a source of energy to grow; whereas others may Indifferent to light.
- Each kinds of microorganisms grows in characteristics manner. e.g. Growth in liquid medium may be abundant; It may be evenly dispersed throughout medium, or it may occur only as a sediment at the bottom or only as thin film or pellicle at the top.
- On solid media ,microbes grow as colonies – distict,compact masses of cells that are macroscopically visible. Colonies are characterized by their size,shape,testure,consistancy, color and other notable features.

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4. Metabolic characteristics

- **The life processes of the microbial cell are a complex integrated series of chemical reactions which is known as metabolism.**
- **Variety of these reactions help to characterize and differentiate various microorganisms.**
- **For example, Some bacteria obtain energy by absorbing light while others oxidize inorganic or organic compounds.**
- **Microorganisms differ in ways in which they synthesize cell components during growth.**
- **The various chemical reactions of an organism are catalyzed by proteins called Enzyme and the enzymes produced by different microorganisms may also vary significantly.**

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5. Genetic characteristics

- DNA of microorganisms contain constant and characteristic features which can help in their characterization.
- These are:

(a) **DNA Base composition**: It is important to note that the DNA molecule is made up of base pairs: guinine-cytosine and adenine-thymine.Of the total number of nucleotide bases present in the DNA that percentage represenred by mol% G+C value. Values for various organisms range from 23 to 75.

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(b) The sequence of nucleotide bases in the DNA: This sequence is unique for each kind of organism and is most fundamental of all characteristics of an organism. It has great significance for microbial classification.

- **The presence of plasmid DNA along with chromosomal DNA can add special characteristics on the cells containing them such as ability to make toxins or to become resistant to antibiotics or to use unusual chemicals as nutrients.**

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6. Pathogenic characteristics

- **The ability to cause disease is characteristic of microorganisms.**

- **Microorganisms can be pathogenic for animals, plants or human and some can cause disease to other microorganisms too. e.g.**
  (1)viruses called bacteriophages can infect and destroy other bacterial cells.
  (2)Bacteria known as bdellovibrios are predatory on other bacteria.

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7. Ecological characteristics

- **Habitat is an important parameter for characterization.**
- **For example, Marine microorganisms are different than those living in fresh water environment.**
- **Microorganisms in the oral cavity are different to those of the intestinal tract.**
- **Some kinds of microorganisms are widely distributed in nature, but others may be restricted to a particular environment.**
- **The relation of an organism to its environment is often complex.**

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8. Antigenic characteristics

- Certain chemical compounds of microbial cells are called antigens.
- Antigenic characteristics has great importance. If microbial cells enter the animal body ,the animal responds to their antigens by forming specific blood serum proteins called antibodies which binds to antigens.
- Antibodies are highly specific for the antigens that induce their formation.
- Antibodies are widely used as tools for the rapid identification of particular kinds of microorganisms.

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- **Antigen –Antibody reaction known as Lock and key model because of the highly specific nature of reaction.**

- **If we know the identity of one part of the system(antigen-antibody) we can identify the other.**

- **For example:**
  If we take typhoid bacterium antibody and mix it with a suspension of unknown bacterial cells, and a positive reaction occurs, we can concluded that the cells are those of the typhoid organism. If no reaction occurs,, then these bacterial cells are of some species other than the typhoid bacterium.

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Microbial Classification, Nomenclature and Identification: (Taxonomy):

Once the characteristics of microorganisms have been determined, the process of classification can begin.

Taxonomy:(Classification of living organisms into groups) is a science of biological classification, consist of tree separate but interrelated parts

(a) Classification – arrangement of organisms into groups(taxa or taxon)

(b) Nomenclature – Assignment of names to taxa

(c) Identification – Determination of taxon to which an isolate belongs

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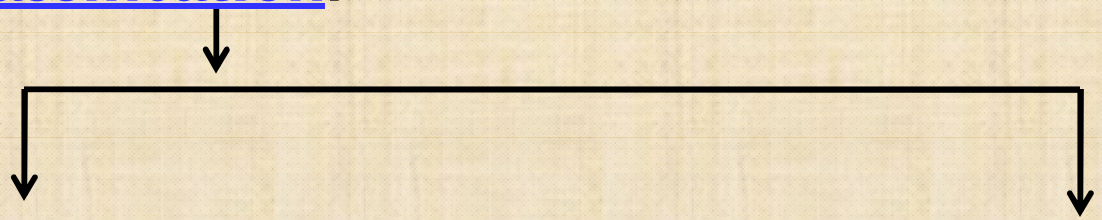
(a) Classification:



Natural Classification:

- Arranges organisms into groups whose members share many characteristics
- This approach to classification does not necessarily provide information on evolutionary relatedness.

Polyphasic Taxonomy:

- Incorporates information from genetic, phenotypic and phylogenetic analysis

Now a days classification is based on polyphasic taxonomy

Phylogenetic Classification System: Groups reflect genetic similarity and evolutionary relatedness .

Phenetic Classification System: Groups do not necessarily reflect genetic similarity or evolutionary relatedness. Instead, groups are based on convenient, observable characteristics.

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Taxonomic Group:

Strain: • A strain is made up of all the descendents of a pure culture.

• It is usually a succession of cultures derived from a initial colony.

Strains: • Strains is vary from each other in many ways i.e. differ biochemically, physiologically, morphologically and differ in antigenetic properties.

• Each strain has a specific history and designation.

For example: Strain ATCC 19554 is a strain of *spirilla* isolated from pond water in Blackburg,Virginia by Wells and Krieg and cultures of the same species that were isolated from other sources would be considered different strains.

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Taxonomic hierarchy is the process of arranging various organisms into successive levels of the biological classification either in a decreasing or an increasing order from kingdom to species and vice versa." Each of this level of the hierarchy is called the **taxonomic category** or **rank**.

Species: Collection of strains that share many stable properties and differ significantly from other groups of strains.
Species is basic taxonomic group.

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Type Strain:

- The type is the strain that is designated to be the permanent reference specimen for the species.
- It is not always the strain that is most typical off all the strains included in the species, but it is the strain to which all other strains must be compared to see if they resemble it closely enough to put into the species.
- Therefore, Type strains are particularly important and special attention is given to their maintenance and preservation ,particularly by ATCC, in the United States or the National Collection of Type Cultures in England.
- Many other culture collection are maintained throughout the world.

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Genus:

- **A collection of similar species is known as genus.**
- **One of the species is designated the type species and this serve permanent example of genus.**

Taxonomic groups of higher rank than genus are listed below:

Family : A group of similar genera
Order : A group of similar families
Class : A group of similar orders
Division : A group of similar classes
Kingdom : A group of similar divisions

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The Goals of Classification:

Taxonomists strive to make classifications that have the following qualities:

(1) Stability: Classification face frequent ,radical changes which lead to confusion. Here every should be made to devise classifications that need only minor changes as new information becomes available.

(2) Predictability: By knowing the characteristics of one member of a taxonomic group, it should be possible to assume that the other members of the same group probably have a similar characteristics,
If this cannot be done ,the classification has little value.

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General Methods for Classifying Bacteria:

Three method are used for classifying bacteria:

(1) Intuitive method
(2) Numerical Taxonomy
(3) Genetic relatedness

(1) Intuitive method:

- **It is rely on the study of the properties of the organisms by taxonomists for several years which decides to represent one or more species or genera.**

Demerit:

- **The characteristics of an organism that is important to one person may not be so important to another.**
- **Different taxonomists arrive at very different groupings.**

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(2) Numerical Taxonomy:

- In these method effort is more objective about grouping bacteria.
- Here scientist may determine many characteristics (usually 100 to 200) for each strain studied, giving each characteristics equal weight.
- Then using a computer he or she calculates the % Similarity (%S) of each strain to every other strain.
  For any two strains, this is:

$$\%S = NS / (NS + ND) \text{ (For 2 strains)}$$

Where, -NS is the number of characteristics that are the same (positive or negative) for the two strains
-ND is the number of characteristics that are different.

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- Those strains having a high %S to each other are placed into groups; those groups having %S to each other are in turn placed into larger groups, and so on.
- The degree of similarity needed to rank a group as species, genus, or other taxon is a matter of judgment for Taxonomist.
- This method of classification has great practical usefulness and also have a high degree of stability and predictability.

Advantages:

- Relatively unbiased.
- Greater degree of stability & predictability.

Disadvantages:

- Only useful within larger groups.
- Exclusively depends upon the mathematical figures plotted on the paper.
- Cannot be related to any particular taxonomic group such as genus or species.

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(3) Genetic relatedness:

- The third and most reliable method of classification is based on the degree of genetic relatedness between organisms.
- This method is most objective and is based on most fundamental aspects of organisms, their hereditary material i.e. DNA
- Development of molecular biology branch of science in1960 provided techniques by which the DNA of one organism could compared with that of other organism. Before that only crude comparison could be made ,based on Mol% G+C values.

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- **It is true that two organisms of the same and similar species that are closely related will have very similar mol% G+C values and It is also true that two organisms having quite different mol% G+C values are not very closely related.**

- **However ,it is important that organisms that are completely unrelated may have similar mol% G+C values.**

- **Therefore, much more precise methods needed by which the DNA molecules from various organisms could be compared with respect to the sequence of their component nucleotides.**
- **This sequence is the most fundamental characteristics of an organism.**
- **Modern technique is available to make such a comparison.**

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- **Basic principle of modern technique can be described by two way :**
  - **(a) DNA homology experiments**
  - **(b) Ribosomal RNA homology experiments and ribosomal RNA oligonucleotide cataloging.**

(a) DNA homology experiments:

- **The ds-DNA molecules from two organisms are heated to convert them to single strands.**
- **The single strands from one organism are then mixed with those from the other organism and allowed to cool.**
- **If the two organisms are closely related, HETERODUPLEXES form. i.e. a strand from one organism will pair with a strand from the other organism.**
- **If the two organisms are not closely related, no heteroduplexes will form.**
- **This method is most useful at the species level classification.**

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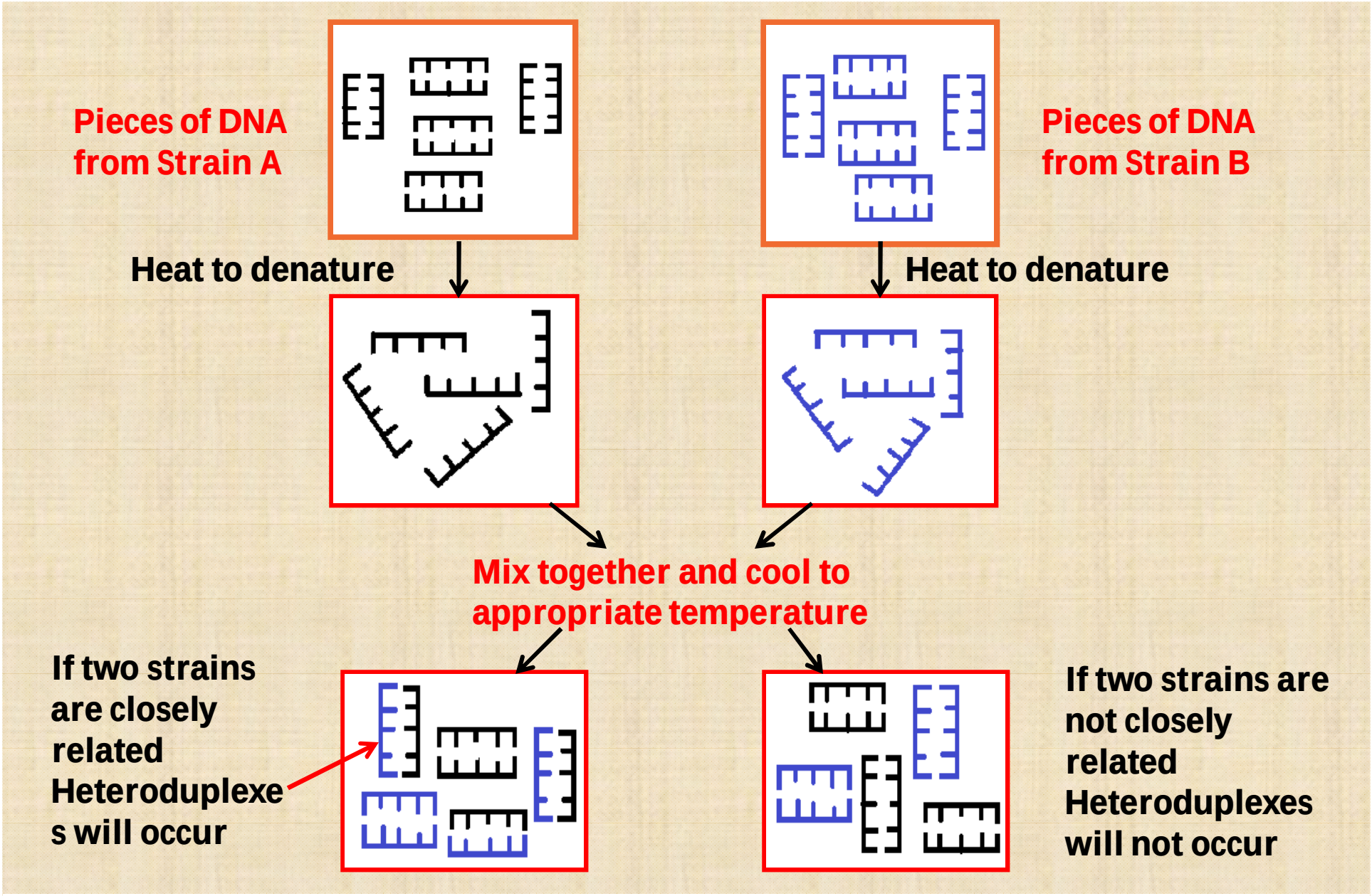

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(b) Ribosomal RNA homology experiments and ribosomal RNA oligonucleotide cataloging :

- **Two organisms which not give a high level of DNA homology, yet they may Still have some degree of relatedness.**
- **Ribosome ,the small granular appearing structure within the cell are composed of proteins and RNA.**
- **This ribosomal RNA (r RNA) is coded for by only a small fraction of the DNA molecule, the r RNA cistrons.**
- **In all bacteria so far studied, the nucleotide sequences of these rRNA genes has been found to be highly conserved , means during evolution the nucleotide sequence of rRNA genes has changed more slowly than that of the bulk of the DNA molecule.**

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- **This means that even if two organisms are only distantly related and show no significant DNA homology they still may be considerably similarity in the nucleotide sequence of their r RNA cistrons.**

- **The degree of similarity that exits can therefore be used as measure of relatedness between organisms, but at a level beyond that of species(at the level of genus, family ,order)' RNA homology experiments and RNA oligo nucleotides cataloging are used to determine the degree of similarity between the r RNA cistrons of different organism.**

- **The techniques are complex and used by only few laboratories.**

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Nomenclature and Identification

Nomenclature:

- **Each species of microorganism has only one officially accepted name by international agreement for precise communication because, If an organism were to be called *Escherichia coli* in one country and *Coprobacterium intestinale* in another, chaos would result. It would be difficult to know same organism was being studied.**
- **The name of a species is merely convenient label, not necessarily descriptive. although some names are,**
  For example:
  (a) *Micrococcus luteus* means "yellow berry" in Latin
  (b) *Proteus vulgaris* is Latin for "common organism of many shapes"
  (c) *Escherichia coli* –the organism of the colon named after Theodor Escherich (German bacteriologist)

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- **Although it might seem that microbial names could be constructed at random but the fact is that certain rules must be followed. Bacteria ,For example are named according to rules set down in the international code of Nomenclature of bacteria.**

- **Carl Linnaeus proposed the system of naming to each organism which is called binomial nomenclature in 1758.**

- **In this system organism are named and their name consist of two parts,**

- **The first part indicate genus and 2nd species from which the organism belong.**

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Rules

- **Each binomial name of an organism consist of two Latin words**
- **1st word indicate genus while 2nd indicate species**
- **The generic name will be start with capital letter and species name with small letter**
- **The binomial name should be Italic or under lined. e.g. *Homo sapiens***
- **Two taxa can not have same name**

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Identification:

- **An organism must be classified before it can be identified.**
- **Here, once the organism is classified a few of its characteristics are selected by which it can be identified by other microbiologists.**
- **In order to be useful identification ,the combination of characteristics chosen which must occur in particular kind of organism and in no other organisms.**
- **The characteristics chosen should be ones that are easy to determine, such as shape, staining reaction and sugar fermentation.**

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For example :DNA homology experiments very useful for classifying an organism, but quite unsatisfactory for the identification of an organism because of the complexity of the procedure.

- Many identification schemes are in the form of keys, which give identifying characteristics arranged in a logical fashion.

- Identification table also useful and generally contain more characteristics than keys, with the information arranged in an easy to read ,summarized form.

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