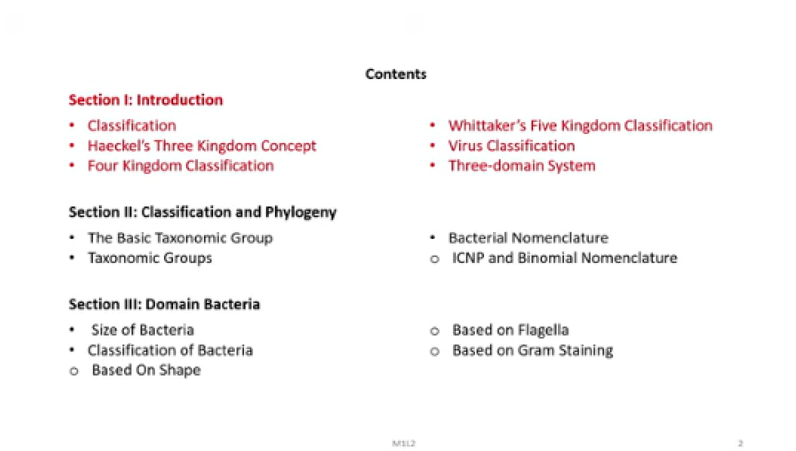
MICROBIAL BIOTECHNOLOGY

Prof. Utpal Bora
Department of Biosciences and Bioengineering
Indian Institute of Technology Guwahati

Lecture02
Lec 2: Classification and taxonomy of microbes A

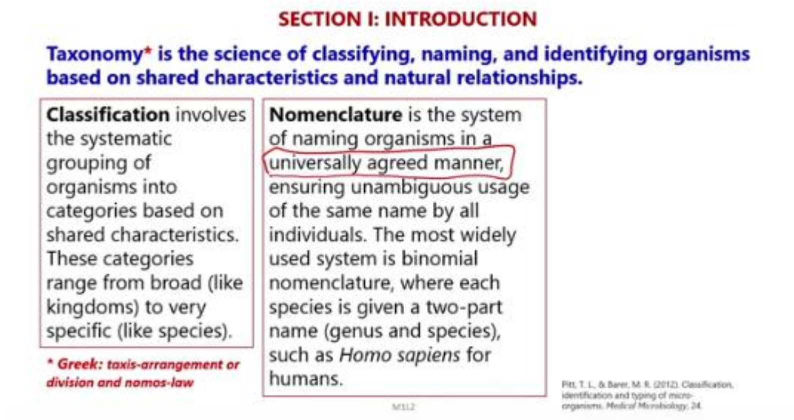
Welcome to my course on microbial biotechnology. We are in module 1, which is basically discussing the introduction and principles of microbial biotechnology, classification and taxonomy of microbes. So, in this lecture number 2, we are going to study about the classification and taxonomy of microbes. So, this particular lecture is divided into the following sections. Section 1 starts with an introduction and gives an idea about the concepts of classification.

Then the various type of classification systems which have been developed over time, like the Haeckel's three kingdom concept, then four kingdom classification, then five kingdom classification. And also, it deals about the virus classification in brief and the three domain system. Then in section two, we'll be studying about the classification and phylogeny. And in section three, we'll be discussing about the particular domain called bacteria. Now, to the beginner, taxonomy is the science of classifying, naming and identifying organisms based on shared characteristics and natural relationship.

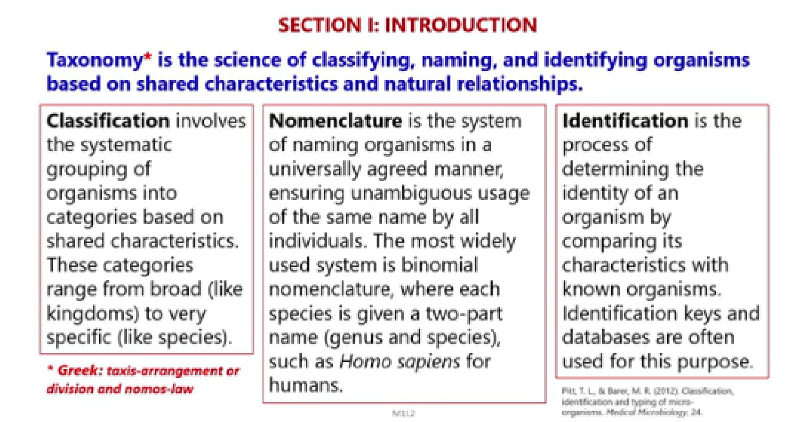


The word taxonomy actually is derived from the two Greek words Taxis which means arrangement or division and Nomos which means law. So, here we have certain guidelines or law which determines how the various microorganisms or living organisms should be arranged systematically. So, now when we tell taxonomy as a discipline, it has various aspects to it. The number one thing is classification.

This involves the systematic grouping of organisms into categories based on shared characteristics. These categories can range from broad like kingdom to very specific like spaces. The second important thing in taxonomy is nomenclature. This is a system of naming organisms in a universally agreed manner. So, otherwise there will be confusion if we give multiple names and these universal agreements ensure unambiguous usage of the same name by all individuals.

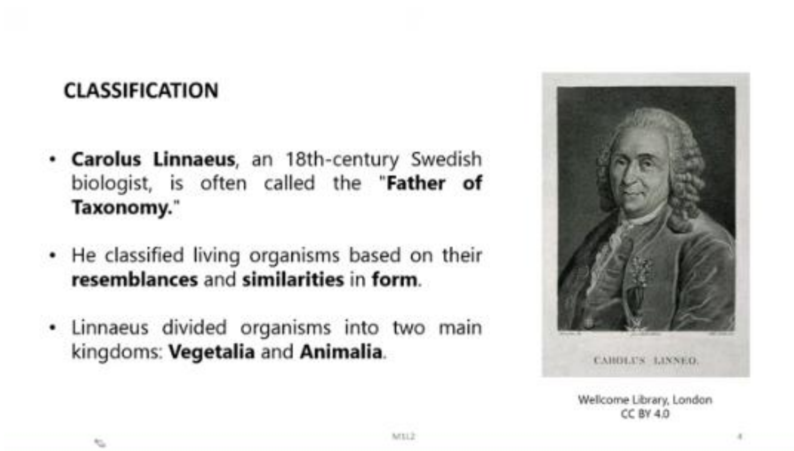


The most widely used system is binomial nomenclature which we will discuss in brief where each species is driven a two-part name, the genus and the species such as homo sapiens for humans. The last important thing in taxonomy is identification. This is the process of determining the identity of an organism by comparing its characteristics with known organisms. Identification keys and databases are often used for this purpose.

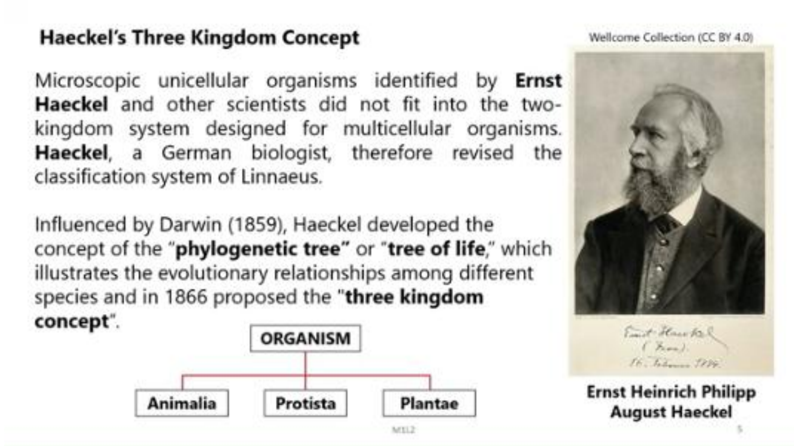


Now, let us start with classification and how the science of classification started. Carolus Linnaeus who was an 18th century Swedish biologist is often called as the father of taxonomy and he classified living organisms based on their resemblances and similarities in form. and similarities in form. So, humans appear similar to apes and then you have cows appearing similar to buffaloes, cats appearing similar to tigers and similarly you have certain plants which have stems and branches and certain plants which do not have stems and branches. So, all these kinds of morphological resemblances and similarities were considered by Carolus Linnaeus and he divided the organisms into two main kingdoms, the vegetalia and the animalia.

Morphological resemblances and similarities were considered by Carolus Linnaeus, and he divided the organisms into two main kingdoms: the Vegetalia and the Animalia. So, that was the simplest classification done by the pioneer of taxonomy. We know that microorganisms have evolved into different forms over time. Similarly, the classification system has also evolved from time to time.

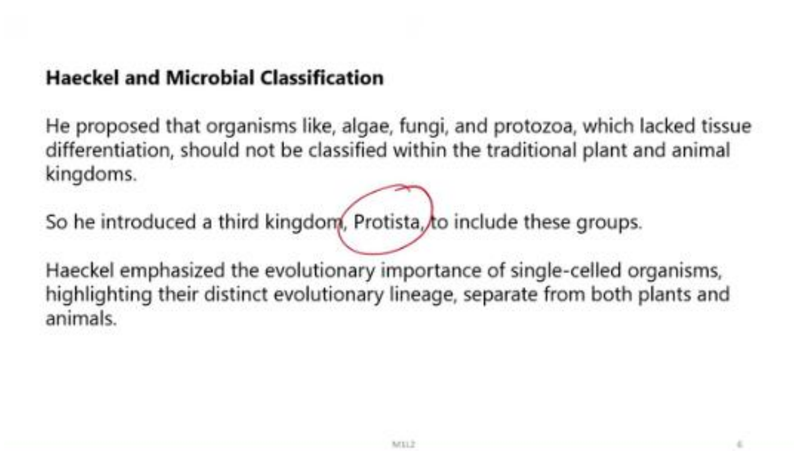


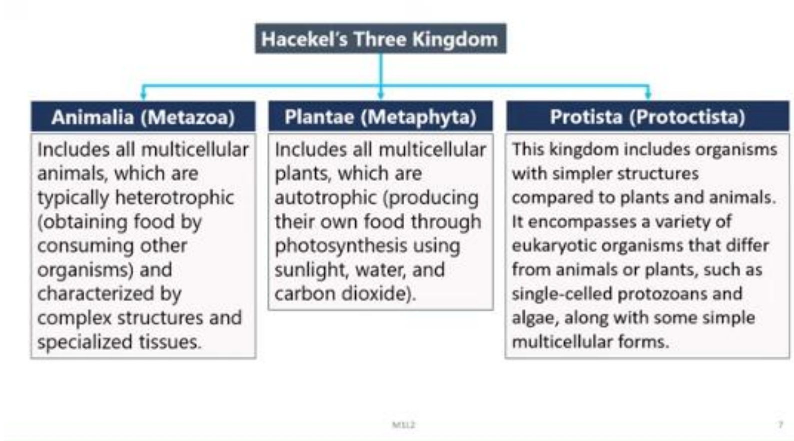
For example, Haeckel put forward a three-kingdom concept. The reason for his expansion of this particular classification was related to microscopic unicellular organisms, which he had identified, as well as other scientists of that time had identified. They found that these did not fit into the two-kingdom system designed for multicellular organisms. So, Haeckel revised this classification system put forward by Linnaeus. Haeckel was influenced by Darwin, and he developed the concept of the phylogenetic tree or tree of life,



which illustrates the evolutionary relationships among different species. In 1866, Haeckel proposed the three-kingdom concept, where he added a new kingdom called Protista, apart from the two proposed by Linnaeus earlier: the Animalia and Plantae. Haeckel proposed that organisms like algae, fungi, and protozoa, which lacked tissue differentiation, should not be classified within the traditional plant and animal kingdoms. So, he introduced the third kingdom, Protista, to include these groups, which, according to him, are different from Plantae and Animalia.

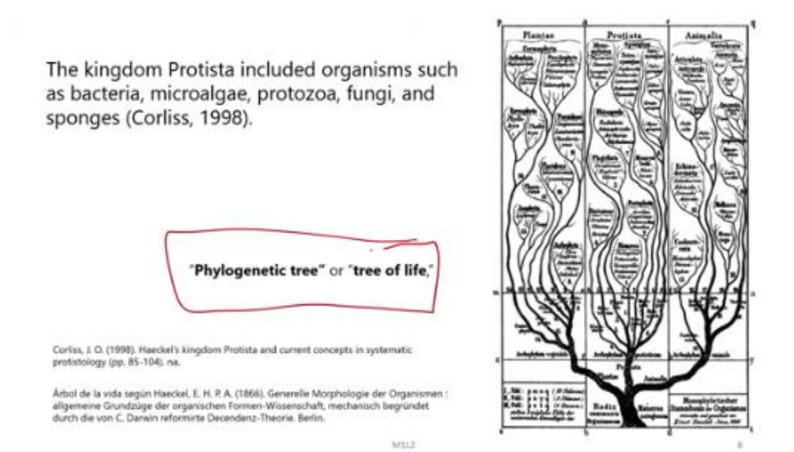
Haeckel emphasized the evolutionary importance of single-cell organisms. They highlighted their distinct evolutionary lineage, separate from both plants and animals. So, in brief, Haeckel's three-kingdom concept can be discussed as follows. As proposed by Linnaeus earlier, the kingdom Animalia includes all multicellular animals, which are typically heterotrophic. That means they obtain food by consuming other organisms.





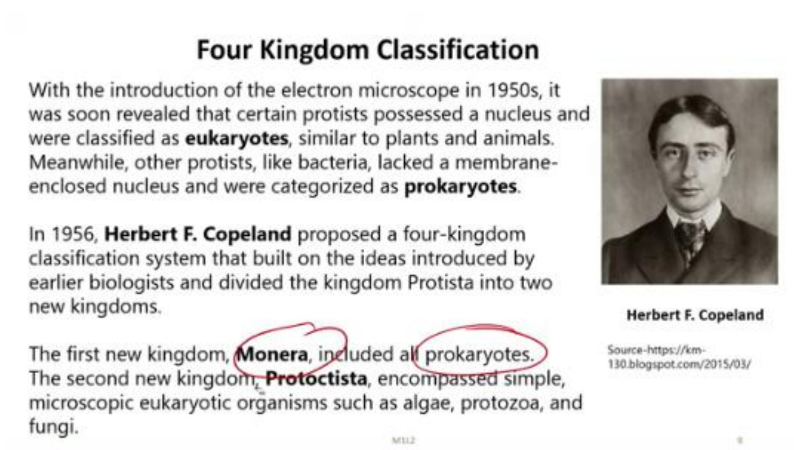
And they are characterized by complex structures and specialized tissues. Then comes the Plantae. These include all the multicellular plants, which are autotrophic. They produce their own food through photosynthesis, using sunlight, water, and carbon dioxide, and they are also called primary producers. Higher animals survive on plants or derive material and energy from plants for their survival. And the third kingdom that was added by Haeckel is the Protista, which is also known as the Protocatista.

This kingdom includes organisms with simpler structures compared to plants and animals, which have complex and specialized tissues. So, these encompass a variety of eukaryotic organisms that differ from animals or plants, such as single-cell protozoans and algae. Along with some other simple multicellular forms. Now, as we have discussed the influence Darwin had on Haeckel and his contribution to science by adding a third kingdom called Protista, as well as putting forward the phylogenetic tree, or the tree of life. You can see here Haeckel's three-kingdom concept, where you have Plantae and Animalia, and here it is the third kingdom, the Protista. Then you have various organisms in all three kingdoms, and they are classified into various branches.



Depending on the level of their similarity in terms of morphology and other features, which were used as keys for their classification. Now, building upon this three-kingdom concept, particularly after the introduction of the electron microscope in the 1950s, a new world was exposed to scientists, and it was soon revealed that certain protists possessed a nucleus. These were classified as eukaryotes, which are similar to plants and animals. Meanwhile, some other protists, like bacteria, lacked a membrane-bound and closed nucleus, and these were classified as prokaryotes. So, in 1956, Herbert F. Copeland proposed a four-kingdom classification system that built on the ideas introduced by earlier biologists.

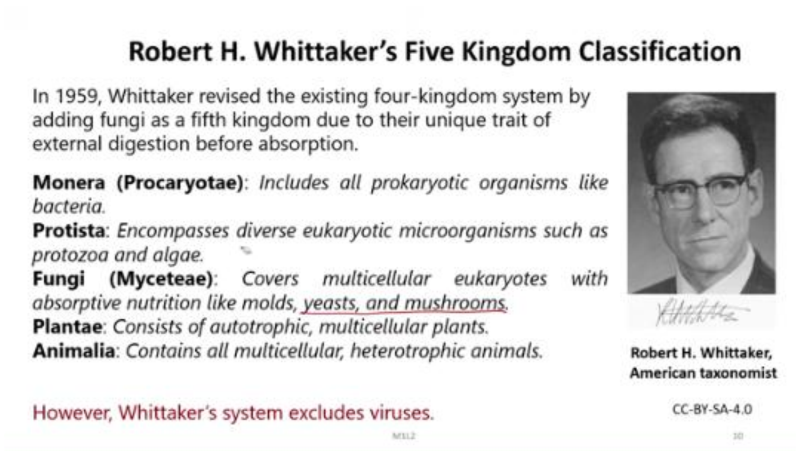
Starting from Linnaeus to others who have contributed. And he divided the kingdom Protista into two new kingdoms. The first new kingdom, Monera, included the prokaryotes. The second new kingdom, Protista, encompassed simple microscopic eukaryotic organisms such as algae, protozoa, and fungi. As I have already mentioned, as organisms have evolved over time, so has their classification system.



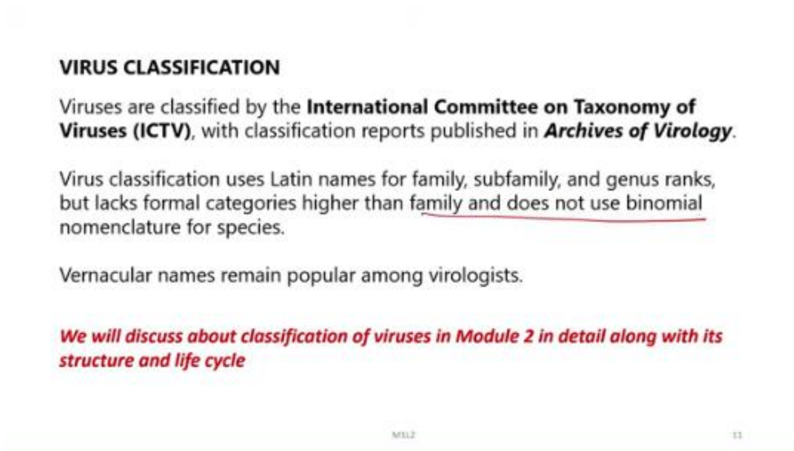
With the advent of newer technologies—for example, when the microscope became available—scientists could see that there are unicellular organisms which are otherwise not visible to the naked eye. So, they included a third kingdom. Then, later on, with the advent of the electron microscope, they could further see that there are some organisms within these microorganisms which have a defined nucleus. They included them in the eukaryotes, and some do not have that defined nucleus, so they kept them as prokaryotes.

Similarly, with the advent of another technology, Whittaker put forward a five-kingdom classification. Here, he particularly added fungi as the fifth kingdom due to the unique trait of external digestion before absorption. So, the metabolism of fungi was unknown earlier or not considered deeply. Whittaker considered this unique feature of these particular organisms, which digest the food outside their body before absorbing it. So, he classified them into Monera, Protista, Fungi, Plantae, and Animalia.

Monera includes all prokaryotic organisms like bacteria. Protista encompasses diverse eukaryotic microorganisms such as protozoa and algae. Then, Fungi covers multicellular eukaryotes. with absorptive nutrition like molds, yeast, and mushrooms. Then, as usual, Plantae consists of autotrophic multicellular plants, and Animalia contains all multicellular heterotrophic animals.

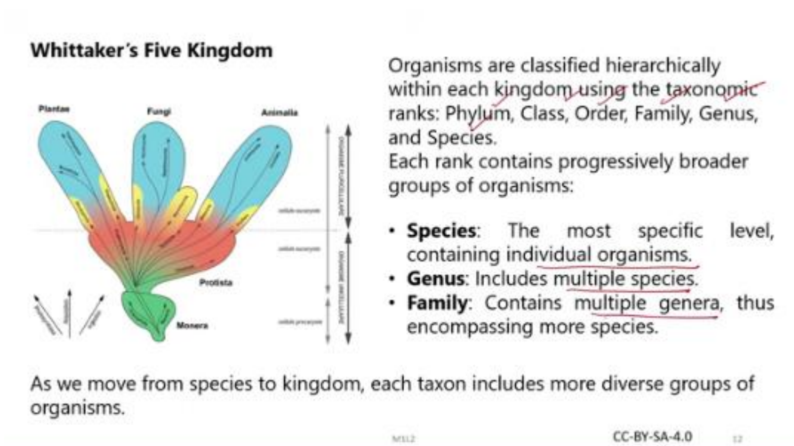


One thing needs to be remembered here: Whittaker's five-kingdom classification did not or could not include viruses within its consideration. So, viruses are classified by the International Committee on Taxonomy of Viruses (ICTV), with classification reports published in Archives of Virology regularly. Virus classification uses Latin names for family, subfamily, and genus ranks but lacks formal categories higher than family and does not use binomial nomenclature for species. Here, vernacular names remain popular among virologists. These being two different subjects, we will discuss the classification of viruses in Module 2 in detail, along with their structure and life cycle.

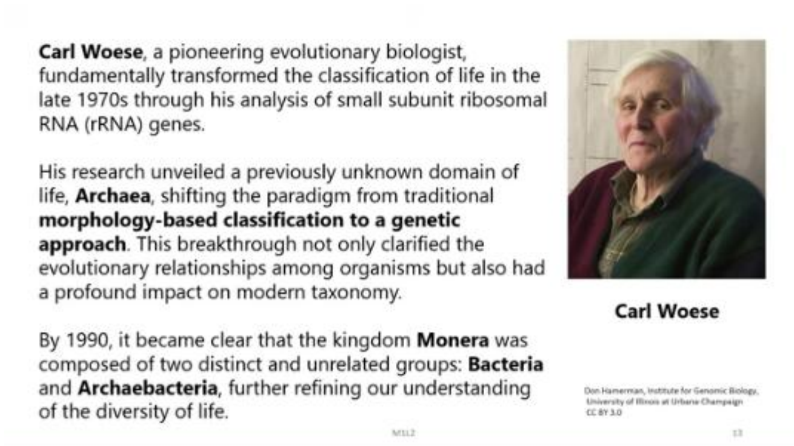


Let us go back to Heidegger's Five Kingdoms. So here you can see the Five Kingdoms: Monera, then Protista, Plantae, Fungi, and Animalia. Now, in this five-kingdom concept, organisms are classified hierarchically within each kingdom using taxonomic ranks like phylum, class, order, family, genus, and species. Each rank contains progressively broader groups of organisms.

For example, species is the most specific level, containing individual organisms. Genus includes multiple species. Family contains multiple genera. Thus, it encompasses more species. So, as we move from species to kingdom, each taxon includes more diverse groups of organisms.

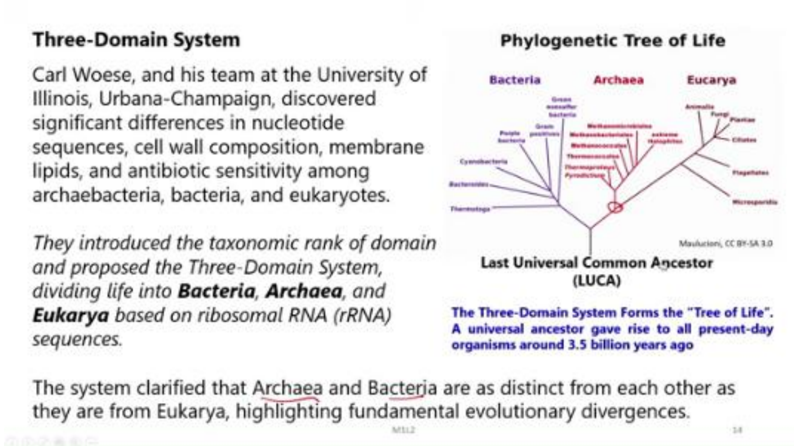


Let us now discuss the work by Carl Woese, who was a pioneering evolutionary biologist and fundamentally transformed the classification of life in the late 1970s through his analysis of small subunit ribosomal RNA genes. The research by Carl Woese unveiled a previously unknown domain of life, known as Archaea, shifting the paradigm from traditional morphology-based classification to a genetic-based approach. This breakthrough not only clarified the evolutionary relationships among organisms but also had a profound impact on modern taxonomy. By 1990, it became clear that the kingdom Monera was composed of two distinct and unrelated groups: Bacteria and Archaebacteria, further refining our understanding of life's diversity.

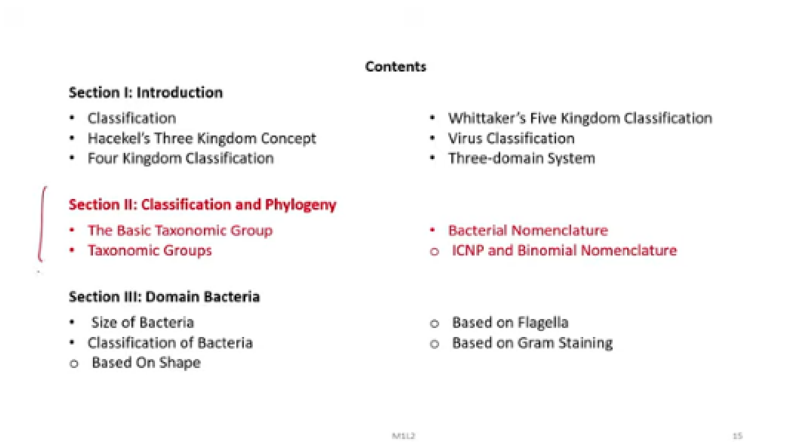


The three-domain system. Carlos and his team at the University of Illinois, Urbana-Champaign discovered significant differences in nucleotide sequences, cell wall composition, membrane lipids, and antibiotic sensitivity among archaea, bacteria, and eukaryotes. They introduced a taxonomic rank of domain. And proposed a three-domain system, dividing life into bacteria, archaea, and eukarya based on ribosomal RNA sequences. The system clarified that archaea and bacteria are as distinct from each other as they are from eukarya, highlighting the fundamental evolutionary divergences. So, all these domains are considered to have a common ancestor from which they evolved, and this is known as the last universal common ancestor or LUCA. From here, you can see that the divergence first was into bacteria and the branch of archaea and eukarya. Then later on, eukarya and archaea diverged during the course of evolution.

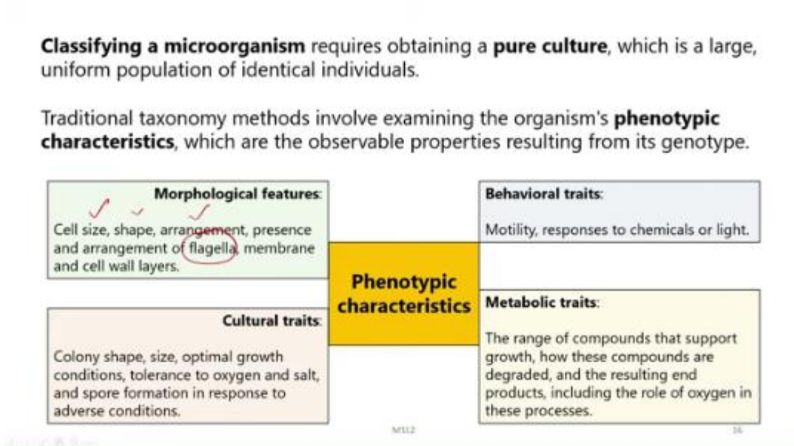
Similarly, in eukarya, we can see the emergence of microsporidia, then flagellates, animals, then fungi, and plantae in the course of evolution. So, if you go into the bacterial domain, there also we can see various kinds of bacteria evolving over the course of time. We will be discussing some of these in the next few slides.



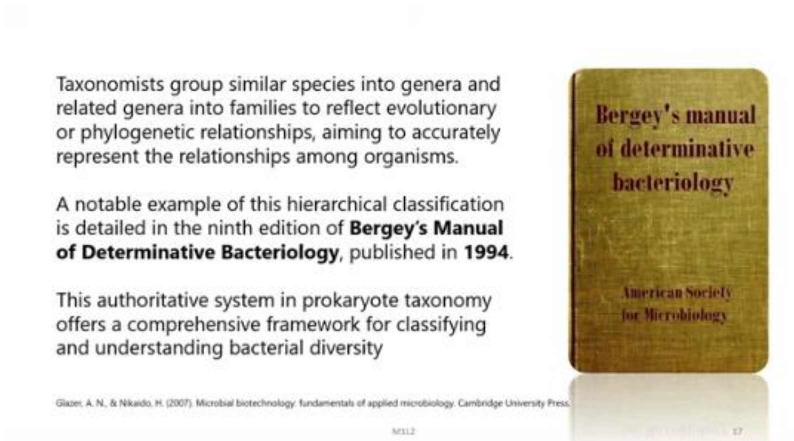
So now let us go to the second section, the classification and phylogeny, where we will discuss the basic taxonomic groups and then the bacterial nomenclature, particularly ICNP and the binomial nomenclature. Now, classifying a microorganism requires obtaining a pure culture, which is a large uniform population of identical individuals. Traditional taxonomy methods involve examining the organism's phenotypic characteristics, or the external characteristics, which are observable properties resulting from its genotype.



So mostly we consider that genotype is a product Sorry, phenotype is a product of the genotype and its interaction with the environment. Now, the various phenotypic characteristics that are considered in this entire process are morphological features, particularly the cell size, the cell shape, the arrangement, presence and arrangement of flagella. membrane and cellular layers. So, while discussing bacteria, we will be discussing all these morphological features one by one.

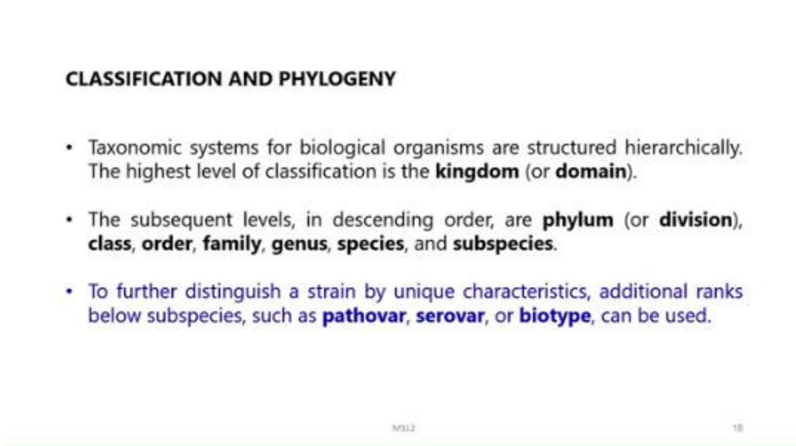


There are things like behavioral traits, particularly motility and responses to chemicals or light, and then metabolic traits, the range of compounds that support growth, how these compounds are degraded, and the resulting end products, including the role of oxygen in these processes. Then another phenotypic characteristic is the cultural trait, which results in the colony shape, size, optimal growth conditions, tolerance to oxygen and salt, and spore formation in response to adverse conditions. Taxonomists group similar species into genera and related genera into families to reflect evolutionary or phylogenetic relationships. aiming to accurately represent the relationships among organisms. A notable example of this hierarchical classification is detailed in the 9th edition of Bergey's Manual of Determinative Bacteriology, published in 1994.

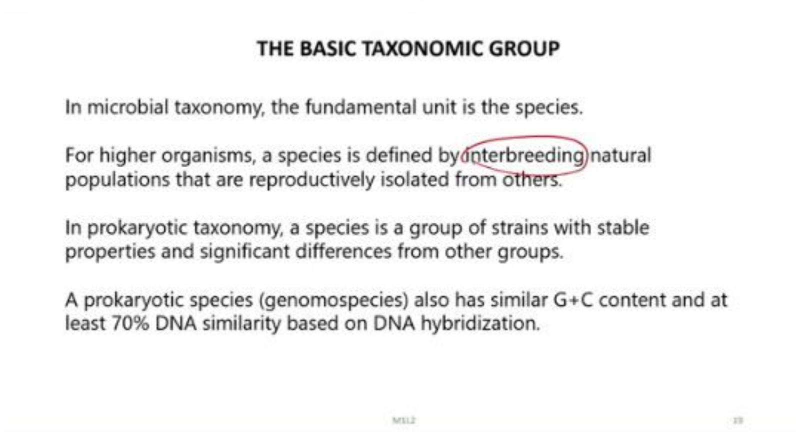


This authoritative system in Prokaryotic Taxonomy offers a comprehensive framework for classifying and understanding bacterial diversity. For those who are more interested in this taxonomic determination of bacteria, please kindly refer to this book. Now, let us discuss the classification and phylogeny. Taxonomic systems for biological organisms are structured hierarchically, as we have already mentioned.

The highest level of classification is the kingdom or domain. The subsequent levels, in descending order, are phylum, class, order, family, genus, species, and subspecies. To further distinguish a strain at the species level by unique characteristics, additional ranks below subspecies, such as pathovar, serovar, or biovar, are also used. The basic taxonomic group in microbial taxonomy, the fundamental unit, is the species.



For higher organisms, a species is defined by interbreeding natural populations that are reproductively isolated from others. In prokaryotic taxonomy, a species is a group of strains with stable properties and significant differences from other groups. A prokaryotic species also has similar G+C content and at least 70% DNA similarity based on DNA hybridization or sequencing data. So,



Briefly, the taxonomic hierarchy is as follows, starting from the species. Below that, we have, of course, the Petrobras and strains, which we are not discussing here. So, a species is a group of related isolates or strains. A genus is a collection of related species. A family is a collection of similar genera.

An order is a collection of similar families. A class is a collection of similar orders. A phylum or division is a collection of similar classes. A kingdom is a collection of similar phyla or divisions. The number of different kingdoms varies depending on the classification system used.

TAXONOMIC GROUPS	
• **Species**: A group of related isolates or strains.	• **Phylum or Division**: A collection of similar classes.
• **Genus**: A collection of related species.	• **Kingdom**: A collection of similar phyla or divisions. The number of different kingdoms varies depending on the classification system used.
• **Family**: A collection of similar genera (suffix -aceae in prokaryotic nomenclature).	
• **Order**: A collection of similar families (suffix -ales in prokaryotic nomenclature).	• **Domain**: A collection of similar kingdoms. Domain is a relatively new taxonomic category that reflects the characteristics of the cells that make up the organism.
• **Class**: A collection of similar orders (suffix -ia in prokaryotic nomenclature).	

Glazer, A. N., & Nikaido, H. (2007). Microbial biotechnology: fundamentals of applied microbiology. Cambridge University Press.

M1L2 20

And finally, a domain is a collection of similar kingdoms. A domain is a relatively new taxonomic category that reflects the characteristics of the cells that make up the organism. A strain is a distinguishable population within a specific taxonomic category. Strains within a species exhibit slight variations from one another. Biovars refer to variant prokaryotic strains distinguished by biochemical or physiological differences.

A **strain** is a distinguishable population within a specific taxonomic category.

Strains within a species exhibit slight variations from one another.

Biovars refer to variant prokaryotic strains distinguished by biochemical or physiological differences.

Morphovars pertain to prokaryotic strains that differ in morphology.

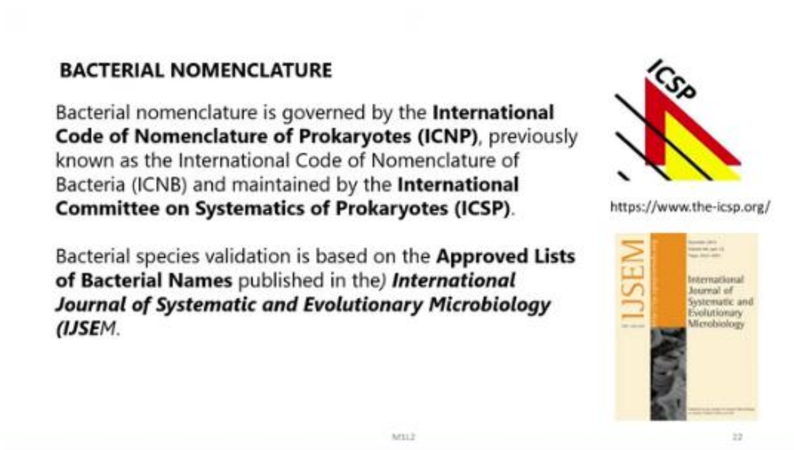
Serovars are prokaryotic strains that differ in serological or antigenic properties.

Biotypes refers to populations or groups of individuals having the same genetic constitution (genotype), e.g.- two biotypes of the cholera bacterium (*Vibrio cholerae)* are *Vibrio cholerae* classic and *Vibrio cholerae* El Tor.

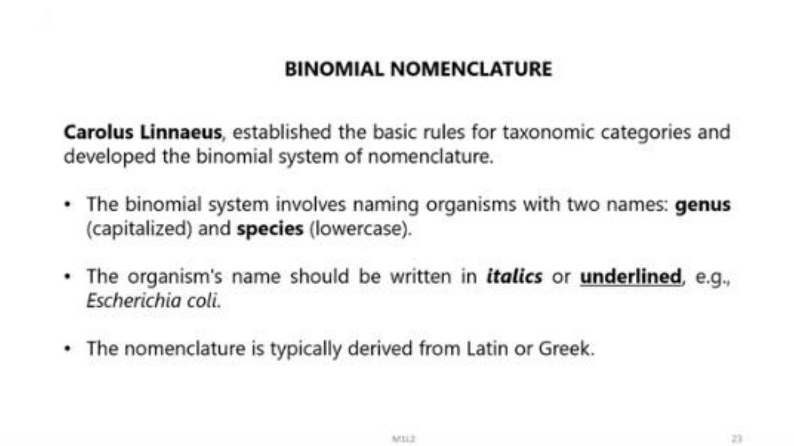
M1L2 21

Morphoverse pertains to prokaryotic strains that differ in morphology. Serophores are prokaryotic strains that differ in serological or antigenic properties. We also sometimes use these as classification tools. Biotypes refer to populations or groups of individuals having the same genetic constitution. For example, two biotypes of the cholera bacterium, Vibrio cholerae, are Vibrio cholerae classic and Vibrio cholerae.

Similarly, there are many such examples. Now, let us learn about bacterial nomenclature. Bacterial nomenclature is governed by the International Code of Nomenclature of Prokaryotes (ICNP), which was previously known as the International Code of Nomenclature of Bacteria (ICNB). This is maintained by the International Committee on Systematics of Prokaryotes (ICSP). Those who are interested can visit this website to learn about the various activities of ICSP.

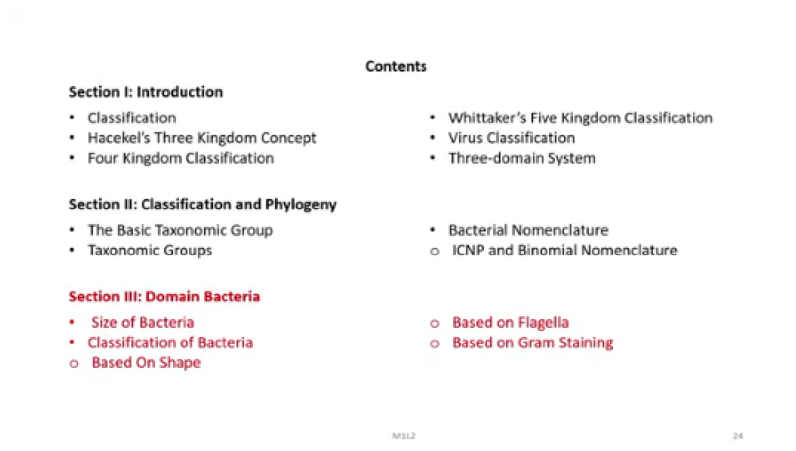


Bacterial species validation is based on the approved lists of bacterial names published in the International Journal of Systematic and Evolutionary Microbiology, and this is a very important step in the nomenclature process. Now, let us discuss binomial nomenclature, and we have already given some examples in the past, such as humans having two parts to their scientific name: Homo sapiens. Carolus Linnaeus established the basic rules of taxonomic categories and developed the binomial system of nomenclature.



The binomial system involves naming organisms with two names. The first is the genus, capitalized, and the species in lowercase. The organisms need to be written in italics or underlined. For example, Escherichia coli. The nomenclature is

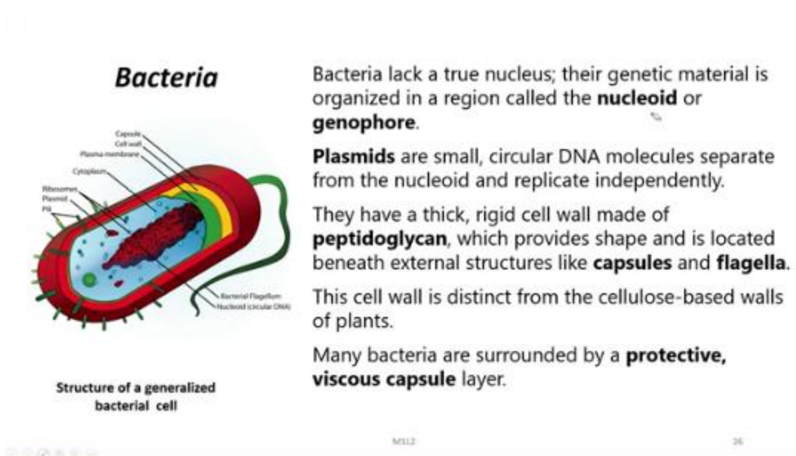
typically derived from Latin or Greek. For example, the name Ascarisia coli is derived from these languages. Now let us discuss one of the important domains: bacteria. Here, we will discuss the size and classification, which is based on flagella and also Gram staining. The domain bacteria.



Antonie van Leeuwenhoek, a Dutch merchant, significantly advanced microbiology by observing bacteria in a toothpick with a self-constructed microscope and published his findings in 'The Secrets of Nature Discovered by Antonie van Leeuwenhoek' in 1695. For this pioneering work, he is often called the father of bacteriology. He referred to these microorganisms as tiny animalcules. The term bacteria, meaning 'small stick,' was introduced by Ehrenberg in 1829 to describe these tiny microorganisms. Bacteria are ubiquitous.

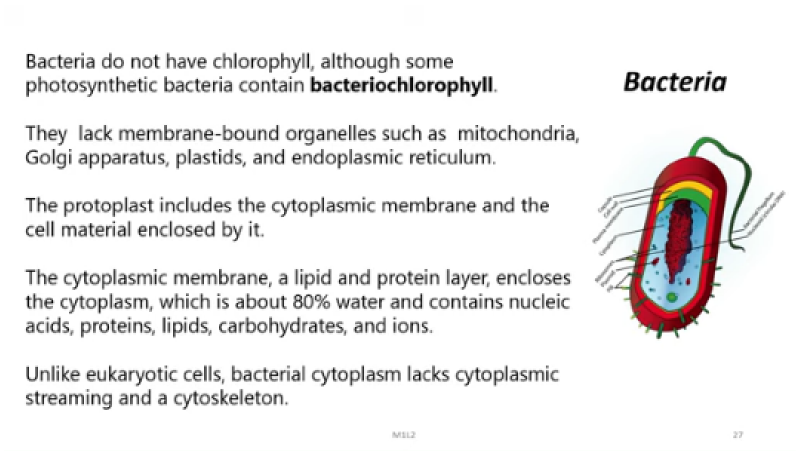
They are found in soil, air, and water. They are unicellular and prokaryotic microorganisms. Bacteria lack a true nucleus. Their genetic material is organized in a region called the nucleoid or genophore. There is another type of genetic material inside a bacterial cell.

These are known as plasmids, which are small circular DNA molecules. These are separate from the nucleoid, and they replicate independently. Bacteria have a thick, rigid cell wall made of peptidoglycan, which provides and is located beneath external structures like capsules and flagella. This cell wall is distinct from the cellulose-based walls of plants.

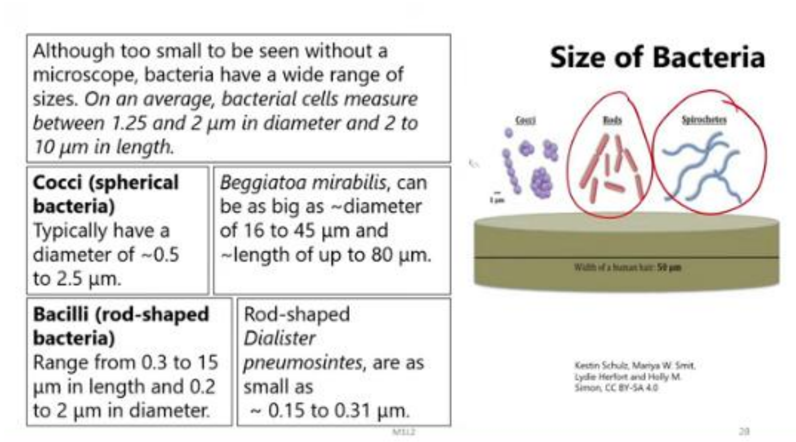


Many bacteria are surrounded by a protective, viscous capsule layer. In this figure, you can see the generalized structure of a bacterial cell, where you have these bacterial flagella, and you have this nucleoid. Apart from this nucleoid, which is genetic material, you have another small genetic material here, which is the plasmid. Then you have The plasma membrane, the cell wall, and the capsule, which is a protective, viscous layer. Bacteria do not have chlorophyll, although some photosynthetic bacteria contain bacterial chlorophyll. They lack membrane-bound organelles such as mitochondria, Golgi apparatus, plastids, and endoplasmic reticulum.

The protoplast includes the cytoplasmic membrane and the cell material enclosed by it. The cytoplasmic membrane, a lipid and protein layer enclose the cytoplasm which is about 80% water and contains nucleic acids, proteins, lipids, carbohydrates and ions. Unlike eukaryotic cells, bacterial cytoplasm lacks cytoplasmic streaming and a cytoskeleton. So, we already know that bacteria are very small and invisible to the naked eye.

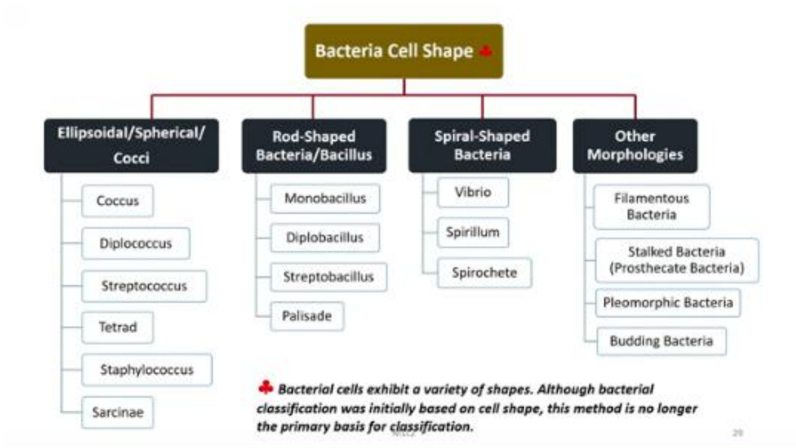


And if we compare the size of various kinds of bacteria with the weight of a human hair which is roughly around 50 microns. We can see that cocci which are round shaped or around roughly around 1 micron or less than that. And then you have the rods over here and the spirochetes which are different kinds of bacteria. So, this is a comparative size analysis of different kinds of bacteria when we compare to the human hair which is visible to us.



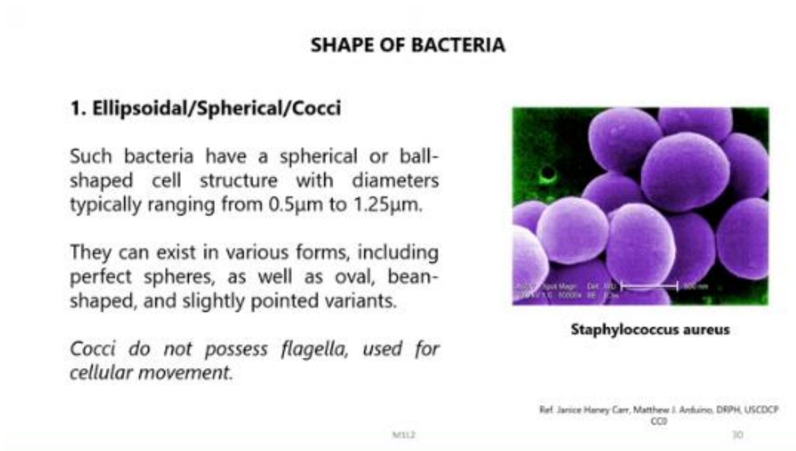
They are no doubt very small in size as an organism but they are not all of the same size. So, bacteria have a wide range of sizes and the average size of a bacterial cell measure between 1.2 and 2 microns in diameter and 2 to 10 micro microns in length and these actually vary from cell types to cell types for example the cocci typically have a diameter of around 0.5 to 2.5 microns and there is an organism of course Begatua mirabilis. This can be as large as 16 to 45 microns in diameter and length up to around 18 micrometer. Similarly, there are Bacillus rotsabit bacteria.

Their size ranges from 0.3 to 15 microns in length and 0.2 to 2 microns in diameter, and there are certain species called Dialister mnemosyne. These are as small as 0.15 to 0.31 microns. Now, we can divide or classify bacteria based on their cell shape because bacterial cells exhibit a variety of shapes. Although bacterial classification was initially based on cell shape, this method is no longer the primary basis of classification. But still, these are used for many practical purposes even today.



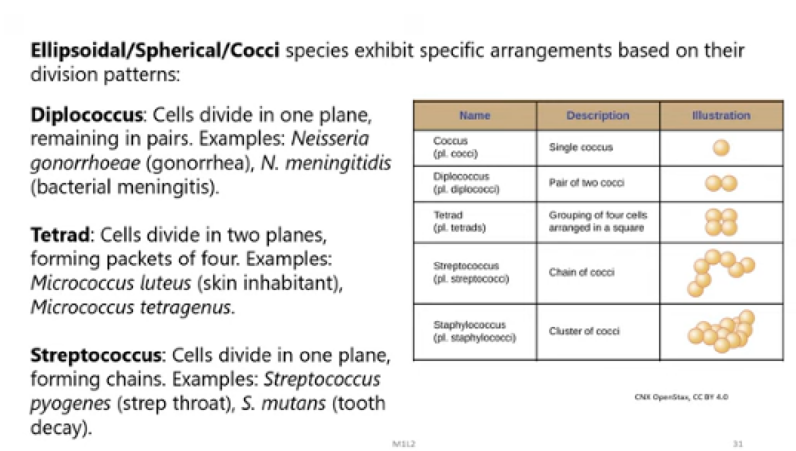
Now, based on their cell shape, the bacteria can be ellipsoidal, spherical, or coccoid. So, and then they can also have many variations like coccus, diplococcus, streptococcus, tetrad, staphylococcus, sarcina. And then they may be rod-shaped, which may be again monobacillus, diplobacillus, streptobacillus, and palisade, and they can be spiral-shaped like vibrio, spirillum, spirochete. And there are other morphologies like filamentous bacteria, stalk bacteria, pleomorphic bacteria, and budding bacteria. So, let us discuss these shapes one by one.

Ellipsoidal, spherical, or cocci. So, you can see from this figure that they are spherical or globular. And you can very well compare their sizes with this scale, which stands for around 500 nanometers. And this is one species, Staphylococcus aureus. This would have been in italics or it should have been underlined as per the binomial nomenclature.



So, these ellipsoidal bacteria have a spherical or ball-shaped cell structure with diameters ranging from around 0.5 microns to 1.25 microns. They exist in various forms, including perfect spheres as well as oval, or they may be bean-shaped, and they may be slightly pointed variations. These cocci do not possess any flagella for cellular movement. These ellipsoidal bacteria exhibit specific arrangements based on their division patterns.

For example, you can see cocci existing as a single cell or single coccus, as you can see in this figure, and then they may also appear in pairs. So we call them diplococci. So when the cells divide in one plane, they remain in pairs. We have many examples of such diplococci, like Neisseria gonorrhoeae, which causes gonorrhea, or Neisseria meningitidis, which causes bacterial meningitis.



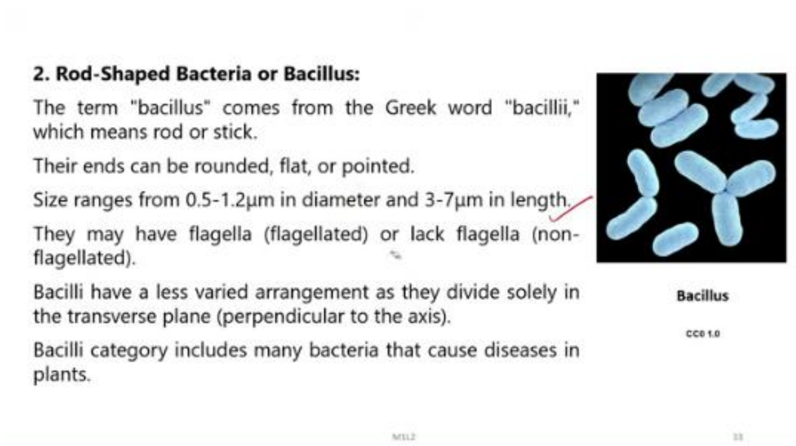
Then we have another arrangement called a tetrad, where you can see four cells arranged in a plane. When the cells divide, they divide in two planes, forming packets of four. You have examples like Micrococcus luteus, which is an inhabitant of the skin. Then you have Micrococcus tetragenus. Then we have streptococci.

This is basically a chain of cocci, if you look at it. These cells divide in one plane. Forming chains. So, you have Streptococcus pyogenes or Streptococcus mutans, which are respectively responsible for strep throat or tooth decay. Another type is the staphylococci.

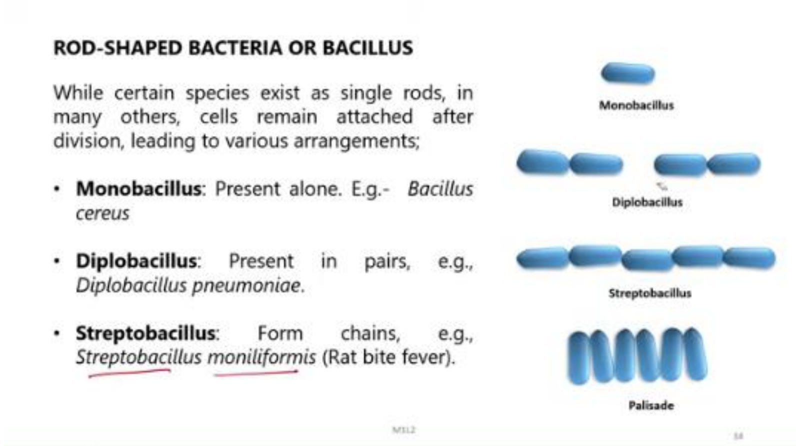
The cells divide in multiple planes in an irregular pattern, forming clusters. Resembling bunches of grapes. Examples of this type are Staphylococcus aureus. It causes food poisoning, toxic shock syndrome, and also skin infections. Then we have sarcinae.

Here, cells divide in three planes in a regular pattern, forming cuboidal or geometric packets, and the most important example here is Sarcina lutea. Now, let us discuss the various forms of rod-shaped bacteria or bacilli. The term bacillus comes from the Greek word 'baktron,' which means rod or stick. So, their ends can be rounded, flat, or pointed, and their size ranges from 0.5 to 1.2 microns in diameter and 3 to 7 microns in length. They may have flagella, so they may be flagellated, or they may lack flagella, meaning they are non-flagellated.

Bacilli have a less varied arrangement as they divide solely in the transverse plane, which is perpendicular to the axis. The bacilli category includes many bacteria that cause diseases in plants. So let us examine some of the forms of rod-shaped bacteria. While certain species exist as a single rod, monobacillus, in many others, the cells remain attached after division, leading to various arrangements.

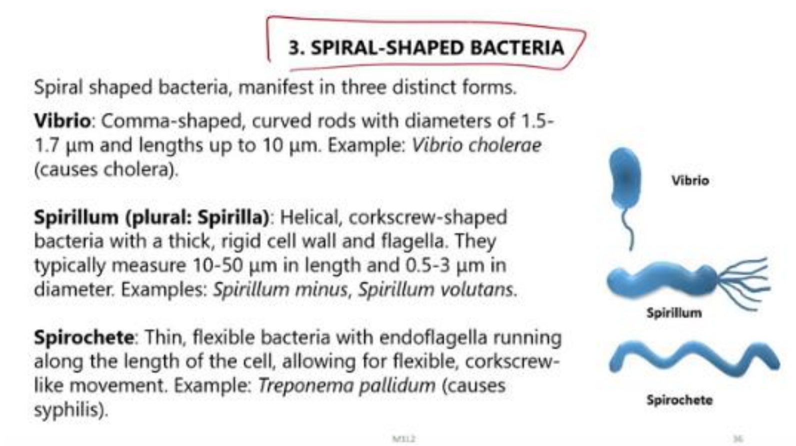


For example, in diplobacillus, like diplococcus, they are present in pairs. For example, Diplobacillus pneumoniae, then you have streptobacillus, which forms chains. For example, Streptobacillus moniliformis. Now, this palisade is also a similar type of arrangement like the chain arrangement, but here, a hinge is formed alternately at one site. So due to the alternate hinge formation,

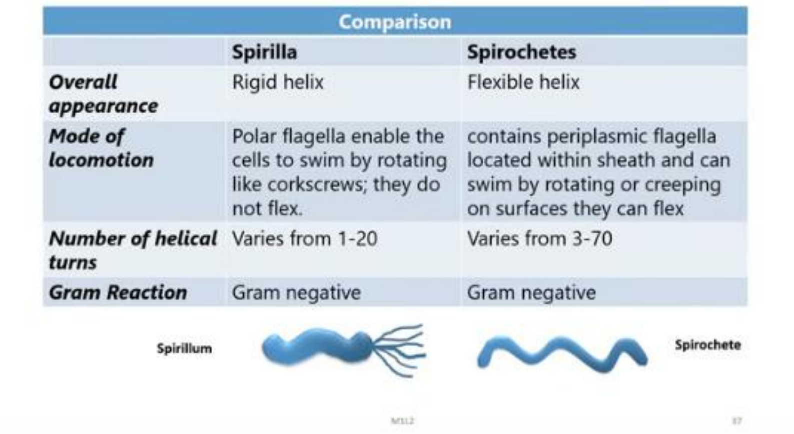


they form chains that are folded in space. So streptobacillus forms chains; an example is Streptobacillus moniliformis, which causes rat-bite fever. Palisade occurs very rarely, resulting in a palisade arrangement. In which cells in a chain are partially attached with a small hinge region, the ends folding or snapping back upon each other, forming a row of cells side by side. Now let us see the various forms of spiral-shaped bacteria.

Spiral-shaped bacteria manifest in three different forms. The first one is the Vibrio. Then you have Spirillum and Spirochete. Vibrio is basically comma-shaped. Curved rods with diameters of around 1.5 to 1.7 microns and lengths up to 10 microns.



The best example is Vibrio cholerae, which causes cholera. Then we have Spirillum, the plural of which is Spirilla. These are helical, corkscrew-shaped bacteria with a thick skin, a rigid cell wall, and flagella. They typically measure 10 to 15 microns in length and 0.5 to 3 micrometers in diameter. Examples include Spirillum minus and Spirillum volutans. Then we have spirochetes, which are thin, flexible bacteria with endoflagella running along the length of the cell, allowing for flexible corkscrew-like movements. An example is Treponema pallidum, which causes the disease syphilis. Now, if you compare Spirilla and Spirochetes, we can see the overall appearance is a rigid helix in Spirilla and a flexible helix in Spirochetes. The mode of locomotion in Spirilla involves polar flagella, meaning the flagella are on one side, enabling the cells to swim by rotating like corkscrews; they do not flex.

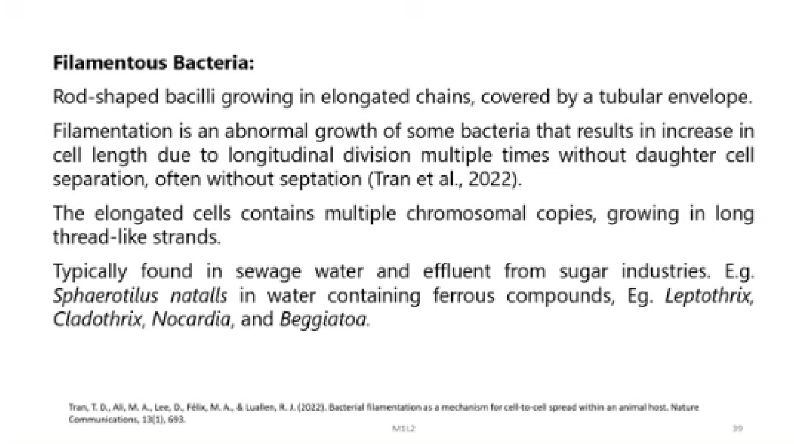


Comparison		
	Spirilla	**Spirochetes**
Overall appearance	Rigid helix	Flexible helix
Mode of locomotion	Polar flagella enable the cells to swim by rotating like corkscrews; they do not flex.	contains periplasmic flagella located within sheath and can swim by rotating or creeping on surfaces they can flex
Number of helical turns	Varies from 1-20	Varies from 3-70
Gram Reaction	Gram negative	Gram negative

In Spirochetes, they contain periplasmic flagella located within the sheath. They can swim by rotating or creeping on surfaces and can flex. The number of helical turns in Spirula ranges from around 1 to 20, and in Spirochetes, it ranges from 3 to 70, which is almost more than 3 times. The Spirula shows a Gram-negative reaction, as well as the Spirochetes, which also show the Gram-negative reaction. What is this Gram-negative reaction? We will discuss it in one slide later. Then there are other morphologies in addition to all these morphologies that we have discussed.

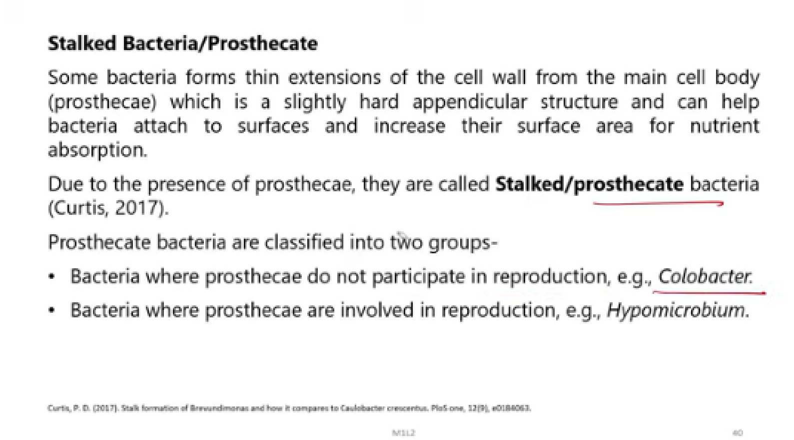
Some bacteria are spindle-shaped or form branching filaments, while certain archaea display square or star-like morphologies. The exact reasons for these shapes are not fully understood, but they are believed to offer adaptive advantages. Some examples of various morphologies that do not fall into the earlier categories are filamentous bacteria, stalked bacteria, or pleomorphic bacteria. Filamentous bacteria are rod-shaped bacilli growing in elongated chains covered by a tubular envelope.

Filamentation is an abnormal growth of some bacteria that results in an increase in cell length due to longitudinal division multiple times without daughter cell separation, often without septation. The elongated cells contain multiple chromosomal copies growing in long thread-like strands. These are typically found in sewage water and effluent from sugar industries. Some examples are Sphaerotilus natans in water containing ferrous compounds, as well as Leptothrix, Cladothrix, Nocardia, and Beggiatoa. Stalked bacteria. Some bacteria form thin extensions of the cell wall.

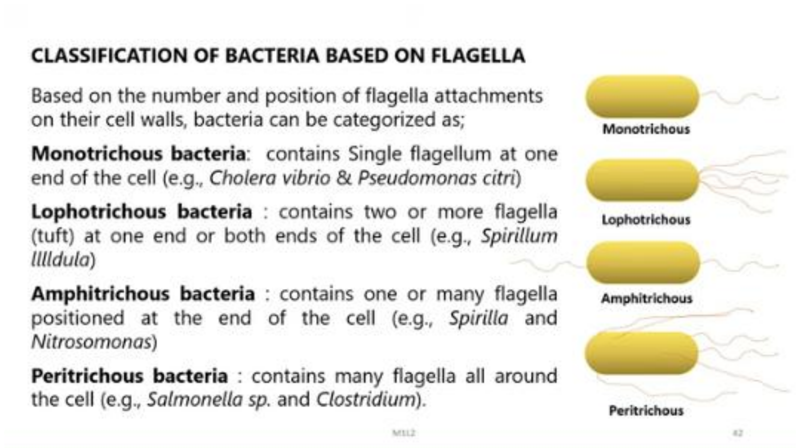


From the main cell body, which is a slightly hard, appendicular structure, and can help bacteria attach to surfaces and increase their surface area for nutrient absorption. Due to the presence of prostheca, they are called stalked or prosthecate bacteria. Prosthecate bacteria are classified into two groups. Bacteria where prostheca do not participate in reproduction. Example, Caulobacter.

And then bacteria where prostheca are involved in reproduction. Example, Hyphomicrobium. Pleomorphic bacteria. Pleomorphic bacteria such as Acetobacter exhibit the ability to alter their shape and structure in response to changes in the environment. These bacteria are found in diverse forms, demonstrating a range of morphological variations.



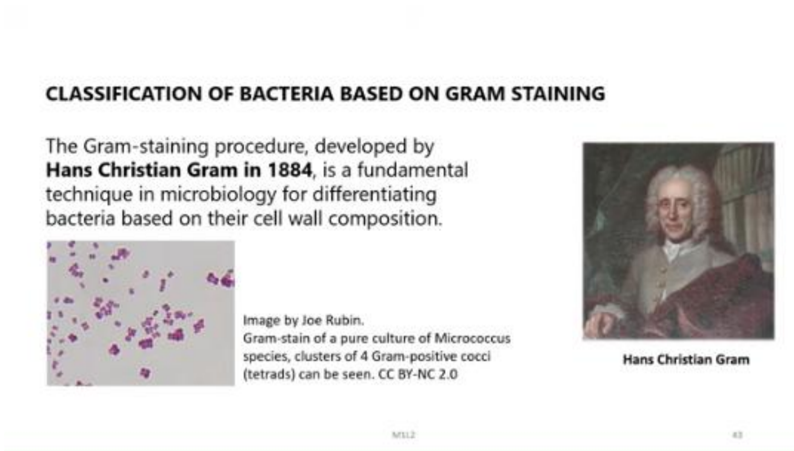
Pleomorphism results from individual variations in cell wall structure due to nutritional or slight hereditary differences. Corynebacterium diphtheriae cells, typically rod-shaped, show diverse forms in culture such as club-shaped, swollen, curved, filamentous, and coccoid. Mycoplasma, lacking cell walls, exhibit extreme variations in shape, showcasing the pinnacle of pleomorphism. Classification of bacteria based on flagella. Based on the number and position of flagella attachments on their cell walls, bacteria can be categorized as monotrichous, lophotrichous, amphitrichous, and peritrichous.



What is monotrichous bacteria? They contain a single flagellum. At one end of the cell. Examples are Vibrio cholerae and Pseudomonas citri. Lophotrichous bacteria contain two or more flagella.

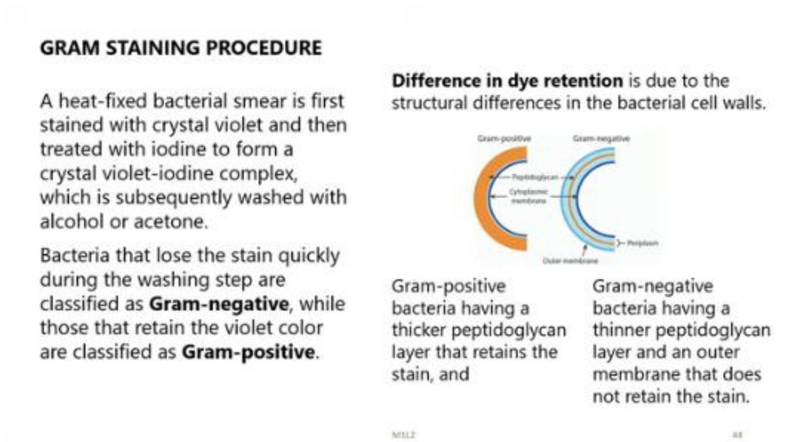
Again, at one end of the cell or sometimes at both ends of the cell. Example, Spirillum volutans. Amphitrichous bacteria contain one or many flagella positioned at the ends of the cell. Example, Spirillum and Nitrosomonas. Then there is peritrichous bacteria which contain many flagella

all around the cell, examples are Salmonella species and Clostridium species. Let us now discuss one of the most important methods of bacterial classification, which is still used particularly in clinics as well as in fundamental basic microbiology research. So this is known as Gram staining or the classification of bacteria based on Gram staining. This method was developed by Hans Christian Gram in 1884 and it is a fundamental technique in microbiology for differentiating bacteria based on their cell wall composition. So here you can see a slide where Gram staining has been done which is of a pure culture of Micrococcus species and you can see a cluster of four Gram-positive tetrads in many places.



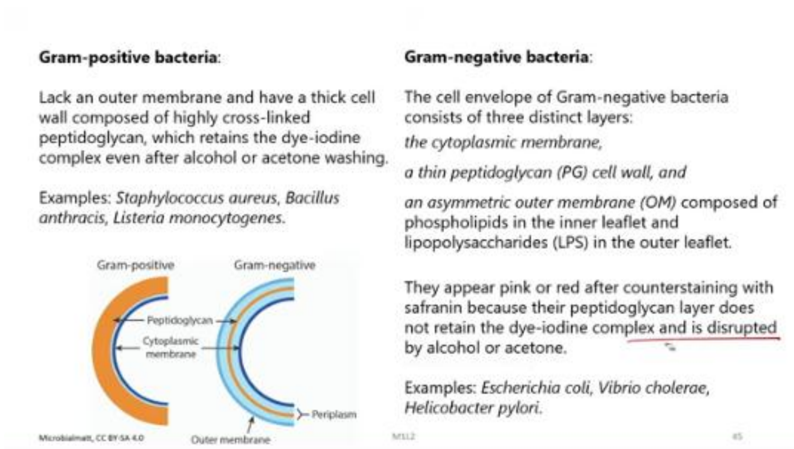
Now, how is this Gram staining done? So, here, a bacterial smear is fixed onto a slide and then stained with crystal violet, followed by treatment with iodine to form a crystal violet-iodine complex. This is subsequently washed with alcohol or acetone. Bacteria that lose the stain quickly during the washing step are classified as Gram-negative, while those that retain the violet color are classified as Gram-positive. Now, why is there a difference in the retention of the dye?

This dye retention is due to the structural differences in the bacterial cell walls. For example, Gram-positive bacteria have a thicker peptidoglycan layer that retains the stain. You can see here the thickness of the peptidoglycan layer, which is quite large in Gram-positive bacteria, while it is very small in Gram-negative bacteria. So, Gram-negative bacteria have a thinner peptidoglycan layer and an outer membrane. As you can see here, that does not retain the stain.



So, it is basically the peptidoglycan layer, which is thick in Gram-positive bacteria, that retains this dye. So, if you look at this diagram once more, you can see it lacks an outer membrane and has a thick cell wall composed of highly cross-linked peptidoglycan. This will retain the dye-iodine complex even after alcohol or acetone washing. Examples of Gram-positive bacteria are Staphylococcus aureus, Bacillus anthracis, and Listeria monocytogenes. And if you look at the cell envelope of Gram-negative bacteria, you can see that it basically has three distinct layers.

The cytoplasmic membrane, the peptidoglycan, cellulose, and an asymmetric outer membrane composed of phospholipids in the inner leaflet and lipopolysaccharides in the outer leaflet. They appear pink or red after counterstaining with safranin because their peptidoglycan layer does not retain the dye. Iodine complex and is disrupted by alcohol or acetone. Examples of gram-negative bacteria are Escherichia coli, which is also a model organism; Vibrio cholerae, which causes cholera; and Helicobacter pylori.



So, with this, we come to the end of lecture number 2, which is basically the taxonomy. And classification. Here, we have discussed at length about the domain bacteria. We will discuss the other domains in some of the future classes. Thank you.

Amen.