### MICROBIAL BIOTECHNOLOGY

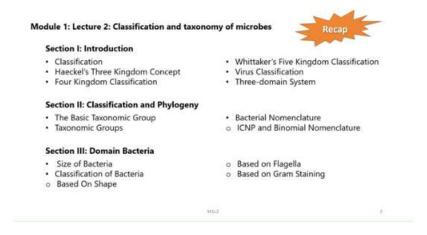
### Prof. Utpal Bora Department of Biosciences and Bioengineering Indian Institute of Technology Guwahati

### Lecture03

### Lec 3: Classification and taxonomy of microbes B

Welcome to my course on microbial biotechnology. We are in module number 1 and today we are going to discuss about classification and taxonomy of microbes. Before we begin, let us have a recap of the last lecture, lecture number 2, which was on the classification and taxonomy of microbes and this particular lecture is also a continuation of that last lecture. There we had studied about various classification concepts starting from the two kingdom to three kingdom to Pythagoras five kingdom classification. And then finally we studied about the classification and phylogeny where we studied about the basic taxonomic unit and the bacterial nomenclature including ICNP and binomial nomenclature.

Then we had some discussion on the domain bacteria. Today we are going to continue the classification of bacteria based on modes of metabolism, then cell wall characteristics and based on RNA sequencing. And then we will go on to discuss about the domain archaea and how it is classified. Then finally, we will also study about the eukaryotic microorganisms like fungi, the protists in today's lecture. Before we begin, let us discuss in brief about various modes of metabolism in organisms.



We know that to support an organism's growth, including humans, we require energy. and reducing power to drive the necessary biosynthetic reactions. Now various organisms do

these energy acquisition by different modes. So some of these are the heterotrophs which use organic compounds as their primary source of cell carbon. Then we have autotrophs which use carbon dioxide as their major source of carbon like cyanobacteria and algae.

### **MODES OF METABOLISM IN ORGANISMS**

To support their growth, all organisms require energy and reducing power to drive the necessary biosynthetic reactions.

Heterotrophs use organic compounds as their primary source of cell carbon.

**Autotrophs** use carbon dioxide as their major carbon source. Eg. Cyanobacteria and algae

Chemotrophs generate adenosine triphosphate (ATP) using chemical bond energy.

Phototrophs produce ATP using light energy.

Then we have chemotrophs that generate energy or ATP using chemical bond energy. Then finally, we have phototrophs, which produce ATP using light energy. Now, these kinds of modes of metabolism can actually be used for classifying organisms, especially bacteria. So, let us study the classification of bacteria based on their mode of metabolism. So, we can typically divide bacteria into two nutritional types.

The first one is autotrophic, and the second one is heterotrophic. Now, what are autotrophic bacteria? They are bacteria that synthesize their own food from simple carbon sources like carbon dioxide and may be further classified into two types. Number one, photosynthetic bacteria, and number two, chemosynthetic bacteria. Photoautotrophic bacteria like cyanobacteria use light as their energy source and carry out oxygenic photosynthesis.

So here, you can see an image—a microscopic image of Cylindrospermum, which is a filamentous cyanobacteria—and you can see that they can actually become very long in the image. These photosynthetic bacteria possess a special type of pigment known as bacteriochlorophyll, which we have mentioned briefly in the last class. They also have other pigments like bacterioverdin and chlorobium chlorophyll. These pigments are located on spiral structures called chromatophores.

Photosynthetic bacteria possess special type of pigment known as bacteriochlorophyll, along with others pigments such as bacteriviridin or chlorobium chlorophyll. These pigments are located on spiral structures called chromatophores. Similar to higher green plants, photosynthetic bacteria synthesize carbohydrates by fixing atmospheric CO2. Chromatium okenii a Gramnegative, purple sulfur bacterial bacterium performs species carry out anoxygenic photosynthesis using hydrogen sulfide (H2S) instead of anoxygenic photosynthesis and thus can thrive in sulfurwater and produce sulfur granules (S) rather than oxygen rich, oxygen-poor gas (thus anoxygenic). environments such as lakes and sediments. Light Adapted from 2H2S + CO2 Carbohydrates +H2O +2S Wikimedia Commons CCA 4.0 MIG

And similar to higher green plants, photosynthetic bacteria synthesize carbohydrates by fixing atmospheric carbon dioxide. Some bacterial species carry out anoxygenic photosynthesis using hydrogen sulfide instead of water and produce sulfur granules rather than oxygen gas. And this is the reason why we call them anoxygenic. So, you can see here the reaction where hydrogen sulfide and carbon dioxide in the presence of light produce carbohydrates and release water and sulfide granules. So, in this picture you can see one species, Chromatium okenii, which is a gram-negative purple sulfur bacterium.

performing anoxygenic photosynthesis. And due to this capability, these okenii can thrive in sulfur-rich, oxygen-poor environments in certain lakes and also in the sediments. So here you can see the sulfur granules which are being produced, and then these are the polysaccharide granules which are being produced in this entire process. Then these are the vesicles which contain quinone, the carotenoid component, and it also has a flagellum as you can see in this diagram. Chemoautotrophic bacteria are different.

They do not use light for energy. Instead, they get energy from inorganic compounds like hydrogen sulfide, ammonia, or iron, which they use to make their own food. So, we have examples of Nitrosomonas bacteria, which convert ammonium ions into nitrate ions and then in the process generate ATP in the presence of oxygen. There is another bacteria called Nitrobacter or Nitrobacter.

Chemoautotrophic bacteria do not use light for energy. Instead, they get energy from inorganic compounds like hydrogen sulfide, ammonia, or iron, which they use to make their own food. Nitrosomonas bacteria ammonium ions (NH4+) into nitrite ions (NO2-) to NITROGEN generate energy (ATP) in the presence of oxygen. CYCLE Nitrobacter bacteria then convert nitrite ions (NO2") into nitrate ions (NO3"), also producing ATP in the process. These reactions play a crucial role in the environment as they are part of the nitrogen https://www.flickr.com/photos/nois professor/25041091764, CC BY 2.0

These convert the nitrate ions into nitrite ions, and in this process, this also produces ATP. These reactions play a crucial role in the environment, as they are part of the nitrogen cycle, as you can see in this diagram. Let us now discuss heterotrophic bacteria. These bacteria cannot fix carbon dioxide, and they obtain their energy and carbon from external sources. There are two types of heterotrophic bacteria.

The first one is the photoheterotrophs. They use light for energy and organic compounds for carbon. Some notable examples are green non-sulfur bacteria and purple non-sulfur bacteria. You have Chloroflexi and Rhodobacter under these two types. Then, another class of heterotrophic bacteria are the chemoheterotrophs.

### (ii) Heterotrophic bacteria They cannot fix CO₂ and obtain their energy and carbon from external sources. Two types of heterotrophic bacteria: Photoheterotrophs: Use light for energy and organic compounds for carbon. Examples; Green nonsulfur bacteria (Chloroflexus aurantiacus) and Purple nonsulfur bacteria (Rhodobacter sphaeroides). Chemoheterotrophs: Obtain both energy and carbon from organic compounds, such as glucose. Those consuming dead organic matter are called saprobes (e.g., Bacillus subtilis). Those feeding on living organic matter, like human tissues, are parasites (e.g., Citrus pathogens). Some form beneficial partnerships with other organisms, known as symbiotic bacteria (e.g., Rhizobia spp.).

The chemoheterotrophs obtain both energy and carbon from organic compounds, such as glucose. Those consuming dead organic matters are called saprophytes—for example, Bacillus subtilis—and those feeding on living organic matter, like human tissues, are parasites—for example, citrus pathogens. Some form beneficial partnerships with other organisms, and this kind of relationship is known as symbiosis. Some examples of

symbiotic bacteria we have here are the Rhizobia species. We can also classify bacteria based on cell wall characteristics. This ninth edition of Bergey's Manual classifies the kingdom of prokaryotes into four major divisions based on cell wall characteristics.

So, you have here the divisions 1, 2, 3, and 4, and they also have some corresponding names like Gracilicutes, which are gram-negative. and thin skin. Then you have Firmicutes, which are gram-positive and have thick and strong cell walls. Then you have Tenericutes and Mendocicutes. Each of these various divisions also has certain classes.

	edition of Bergey's f all characteristics:	Manual (198	4) classifies the Kingdom Procaryotae into four major divisions based
DIV. NO.	DIVISION NAME	CLASS NO.	CLASS NAME
1	Gracilicutes:  Gram-Negative Bacteria which are thin-skinned	1	Scotobacteria – Gram-negative, non-photosynthetic bacteria.
		ш	Anoxyphotobacteria – Gram-negative, photosynthetic bacteria that do not produce oxygen (includes purple and green bacteria).
		ш	Oxyphotobacteria – Gram-negative, photosynthetic bacteria that produce oxygen (includes cyanobacteria).
II (c	Firmicutes -	L	Firmibacteria – Gram-positive, typically rod-shaped or cocci bacteria.
	Gram-Positive Bacteria which are thick and strong	II	<b>Thallobacteria</b> – Gram-positive, branching filamentous bacteria (includes actinomycetes).
m	Tenericutes	1	Mollicutes – Bacteria lacking a cell wall (includes Mycoplasma).
IV	Mendosicutes	E	Archaebacteria – Bacteria with unique cell wall and membrane compounds (now more commonly referred to as Archaea).

For example, division number one has three classes: one, two, and three. And their corresponding class names you can see are Scotobacteria, which are gram-negative, non-photosynthetic bacteria. Then you have Anoxyphotobacteria, which are also gram-negative, photosynthetic bacteria. that do not produce oxygen. This includes the purple and green bacteria.

Then you have Oxyphotobacteria. These are gram-negative, photosynthetic bacteria that produce oxygen, and this includes the cyanobacteria. Under Firmicutes, we have two divisions. The first one is Firmibacteria, which are gram-positive, typical rod-shaped or cocci bacteria. Then you have the Thalobacteria, which are gram-positive, branching filamentous bacteria.

This includes the actinomycetes. And under the divisions, tenericutes, we have only one class which is mollicutes. These bacteria lack a cell wall and includes microplasma. Similarly, we have Mendocin Q test only one class which is known as the archaea bacteria. These are bacteria without which unique cell wall and membrane compounds.

These are more commonly referred to as archaea currently. Interesting classification of modern times is the classification of bacteria based on RNA sequences. Prior to these,

DNA-DNA hybridization was considered as the gold standard for bacterial species delineation, which measures genetic relatedness by re-associating DNA strands from different organisms. DNA delineation hybridization offers high precision in defining species with 70% or more DNA relatedness and a melting temperature of around 5 degree centigrade or less making it ideal for resolving taxonomic uncertainties. For this reason, it is called as gold standard for bacterial species delineation.

### CLASSIFICATION OF BACTERIA BASED ON rRNA SEQUENCING

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However, despite its accuracy, this method is **labor-intensive**, **time-consuming**, **and costly**, leading to a decline in its routine use as more practical techniques, like 16S rRNA gene sequencing, have become widespread.

New classification schemes based on rRNA sequencing have revealed additional branches in the bacterial "tree."

Some of these align with Bergey's system (e.g., gram-positive bacteria), while others, such as the spirochetes, are genetically distinct and placed into separate categories.

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However, despite its accuracy, DNA-DNA hybridization is a labor-intensive, time-consuming, and costly procedure. And therefore, this has led to a decline in its routine use as more practical techniques like 16S RNA gene sequencing have become available and widespread. Therefore, new classification schemes based on RNA sequencing have revealed additional branches in the bacterial tree. And some of these align with Barge's system. Example, gram-positive bacteria, while others such as the spirochetes are genetically distinct and placed into separate categories.

Why has the 16S rRNA gene become the most widely used genetic marker for studying bacterial phylogeny and taxonomy? There are several factors responsible for this. Number one, the 16S rRNA gene is present in nearly all bacteria, often in the form of multigene families or operons. This particular gene has a conserved function over time, making random sequence changes a more reliable indicator of evolutionary history. It also has adequate length, roughly around 1500 base pairs, which makes it well-suited for bioinformatics analysis.

The 16S rRNA gene has become the most widely used genetic marker for studying bacterial phylogeny and taxonomy due to several factors:

- (i) its presence in nearly all bacteria, often in the form of multigene families or operons:
- (ii) its conserved function over time, making random sequence changes a more reliable indicator of evolutionary history; and
- (iii) its adequate length (1,500 bp), which makes it well-suited for bioinformatics analyses.

Currently, there are approximately 100 recognized bacterial phyla identified through culturing or nucleotide sequencing methods. Let us see some of the prominent phyla and additional groups in the future slides. So, let us start with Proteobacteria. It comes from the word 'proteo,' meaning 'first,' and it is the largest and most diverse bacterial phylum, including many well-known Gram-negative genera like Escherichia coli. It is subdivided into alpha, beta, gamma, delta, and epsilon.

Here, you can see an example of Rickettsia under the microscope. Some of the notable pathogens under these are enteric pathogens like E. coli and its relatives. Then you have Shigella and Salmonella. and Vibrio cholerae, which causes gastroenteritis and cholera. Other significant genera are Neisseria gonorrhoeae, which causes gonorrhea, and Yersinia pestis, which causes bubonic plague. Then you have Rickettsias, which are intracellular parasites transmitted by arthropods like ticks, lice, fleas, and mosquitoes, causing diseases such as Rocky Mountain spotted fever.

### Notable pathogens:

Enteric pathogens: Escherichia spp., Shigella, Salmonella, Vibrio cholerae (causing gastroenteritis and cholera).

Other significant genera: Neisseria gonorrhoeae (causing gonorrhea) and Yersinia pestis (causing bubonic plague).

**Rickettsias:** Intracellular parasites transmitted by arthropods (e.g., ticks, lice, fleas, mosquitoes), causing diseases such as Rocky Mountain spotted fever (*Rickettsia rickettsii*).



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Then we have actinobacteria. The phylum includes gram-positive bacteria with high guanine and cytosine content in their DNA. Many species exhibit branched filamentous growth similar to fungi, although not all do so. Their habitat, particularly, they are found in soil. Actinobacteria are important decomposers, and some species are also aquatic.

Their function includes mostly being non-pathogenic, but there are certain pathogenic genera, which include microbacterium. Some of the famous examples are streptomyces, which produces over two-thirds of naturally derived antibiotics, such as streptomycin and tetracycline, making it a very important species in general. Then we have mycobacterium. This includes pathogens like Mycobacterium tuberculosis and Mycobacterium leprae, which are known for their waxy cell walls containing mycolic acids. And something very interesting: this Actinobacteria contains problematic



bacteria as well as bacteria that provide solutions to this problem. For example, it has disease-causing pathogens, and it also has bacteria that produce antibiotics. The next in line is cyanobacteria. These are prokaryotic bacteria that can exist in unicellular, filamentous, or colonial forms. Historically, they were referred to as blue-green algae due to their pigmentation.

**Cyanobacteria** are prokaryotic bacteria that can exist in unicellular, filamentous, or colonial forms.

Historically referred to as blue-green algae due to their pigmentation, they can also appear black, yellow, green, or red, depending on their pigments.

For example, the presence of red pigments in some cyanobacteria contributes to the periodic reddish discoloration observed in the Red Sea, particularly during blooms of specific species.



TEM image of Prochlorococcus marin a marine evanohacterium

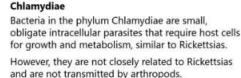
Suke Thompson from Chisholm Lab and Nikki Watson

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They can also appear black, yellow, or green. or red, depending on their pigments. For example, the presence of red pigments in some cyanobacteria contributes to the periodic reddish discoloration observed in the Red Sea, particularly during blooms of certain species. Cyanobacteria are notable among bacteria for their ability to perform photosynthesis using chlorophyll a, similar to algae and green plants, converting carbon dioxide into biomass. They played a crucial role in oxygen evolution around 3 billion years ago, significantly altering Earth's atmosphere by producing oxygen.

So, in the introduction yesterday, we were speaking about the role of microbes in climate change, and we can see that in the past, microbes have played a crucial role in changing the climate of our planet. It is believed that chloroplasts originated from an endosymbiotic event involving a cyanobacterial ancestor. Notable genera include Nostoc, Oscillatoria, and a couple of others. Here, we can see a tiny image of a marine cyanobacterium. Let us now discuss chlamydia.

Bacteria in this phylum are small, obligate intracellular parasites that require host cells for growth and metabolism, similar to rickettsias. However, they are not closely related to rickettsias and are not transmitted by arthropods. Initially, chlamydia was mistaken for viruses due to their small size and parasitic lifestyle. Chlamydia are now recognized as prokaryotic bacteria with a gram-negative cell structure. They reproduce through binary fission, confirming their classification as bacteria, as opposed to viruses.

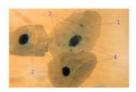


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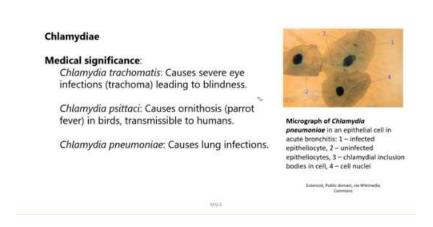


Micrograph of Chlamydia pneumoniae in an epitheliai cell in acute bronchitis; 1 – infected epitheliocyte, 2 – uninfected epitheliocytes, 3 – chlamydiai inclusion bodies in cell, 4 – cell nuclei

Estensist, Public domain, via Willimedia

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There is a micrograph of chlamydia pneumoniae over here in an epithelial cell in acute bronchitis which is number one shows the infected, number two shows the uninfected. And the chlamydial inclusion in number 3 and you can see the cell nuclei in number 4. So chlamydia has a lot of medical significance. For example, chlamydia trachomatis causes severe eye infections leading to blindness. Chlamydia catechi causes ornithosis or fever in birds and this is transmissible to humans.



So, it's kind of a genetic disease. Then we have chlamydia pneumonia which causes lung infections. Spirochetes. The phylum Spirochetes includes over 340 described species, most of which are gram-negative. Spirochetes are characterized by the helical shape and corkscrew movement.

And you can see this helical structure over here in this electron micrograph of Tryponema pallidum. They inhabit diverse environments from mud and sediments to insect digestive tracts and can be pathogenic in the urogenital tracts of vertebrates. Human pathogens include Tryponema pallidum and you can see the picture electron micrograph of this

particular pathogen here and this causes syphilis and certain Borrelia species which are responsible for Lyme disease and relapsing fever. Borrelia species are transmitted by ticks or lice.

### **Spirochaetes**

The phylum **Spirochaetes** includes over 340 described species, most of which are Gram-negative. Spirochetes are characterized by their helical shape and corkscrew movement.

They inhabit diverse environments, from mud and sediments to insect digestive tracts, and can be pathogenic in the urogenital tracts of vertebrates.

Human pathogens include *Treponema pallidum*, which causes syphilis, and certain *Borrelia* species, which are responsible for Lyme disease and relapsing fever. *Borrelia* species are transmitted by ticks or lice.



Electron micrograph of Treponemo

Photo credit Content providers(s); CDC / Dr. David

Bacteroidetes are members of the Dysphyllum, are gram-negative bacteria with some species possessing flagella for mobility. In the human gastrointestinal tract, Bacteroidetes and Firmicutes are the two most dominant phyla, often constituting a significant majority of the microbiome. Bacteroidetes are crucial for breaking down proteins and carbohydrates, producing essential nutrients and energy. They also influence immune function and overall intestinal health. Thus, they are very important as gut microbiota.

One of the notable species is Porphyromonas gingivalis. This species is associated with periodontal disease. Although 16S rRNA is a very promising technology for classification, it has certain limitations. Let us try to understand what these limitations of 16S rRNA gene sequencing classification methods are. There is no doubt that it is a very valuable tool for bacterial classification, particularly in delineating species, but it has limited power at the species level and poor discriminability for some genera.

### Bacteroidetes

Members of the phylum **Bacteroidetes** are **Gramnegative bacteria**, with some species possessing flagella for motility.

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One notable species, **Porphyromonas gingivalis**, is associated with periodontal disease.



Bacteroides biacuti

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This is not very specific for every genus; only in particular genera does it have very poor resolution. High sequence similarity does not always ensures accurate identification. This is something important to remember. For example, in Bacillus globisporus and Bacillus psychrophilus, we have over as high as 99.5% 16S rRNA sequence similarity, but only 23 to 50% DNA relatedness.

Similarly, in Edward Ciela, we have species with around 99.35% to 99.8% 16S RNA similarity, but their DNA relatedness ranges from only 28% to 50%, even though they are distinguishable biochemically. So, several bacterial groups, such as Enterobacteriaceae and rapid-growing mycobacteria, also encounter these resolution issues, emphasizing the need for DNA relatedness studies to overcome taxonomic challenges. So, while using tools like 16S RNA, we should be a little cautious and always look for DNA relatedness in parallel experiments. So, this table lists some examples of bacterial genera and species with identification problems when using 16S RNA gene sequencing.

### Resolution Limits of 16S rRNA gene sequencing

Although 16S rRNA gene sequencing is valuable for bacterial classification it has limited phylogenetic power at the species level and poor discriminatory ability for some genera. High sequence similarity does not always ensure accurate identification.

For example, *Bacillus globisporus* and *Bacillus psychrophilus* have >99.5% 16S rRNA sequence similarity but only 23 to 50% DNA relatedness. Similarly, *Edwardsiella* species show 99.35 to 99.81% 16S rRNA similarity, but their DNA relatedness ranges from 28 to 50%, even though they are distinguishable biochemically.

Several bacterial groups, such as Enterobacteriaceae and rapid-growing mycobacteria, also encounter these resolution issues, emphasizing the need for DNA relatedness studies to overcome taxonomic challenges.

J Clin Microbiol. 2007 Jul 11;45(9):2761-2764.

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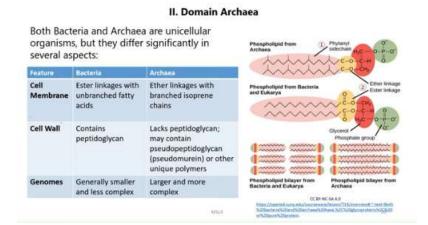
So, you have various genera like Aeromonas, Bacillus, Bordetella, Burkholderia, and Campylobacter. And then, some of these genera have many species that suffer from these kinds of limitations, like in Bacillus: anthracis, cereus, globisporus, and psychrophilus. Let us now move to the next section, Section 2, and discuss the classification of Archaea. So, the domain Archaea. Both Bacteria and Archaea are unicellular organisms, but they differ significantly in several aspects.

Table: Few examples of bacterial genera and species with identification problems using
16S rRNA gene sequencing (Adapted from J Clin Microbiol. 2007 Jul 11;45(9):2761-2764.

Genus	Species	
Aeromonas	A. veronii	
Bacillus	B. anthracis, B. cereus, B. globisporus, B. psychrophilus	
Bordetella	ella B. bronchiseptica, B. parapertussis, B. pertussis	
Burkholderia	B. cocovenenans, B. gladioli, B. pseudomallei, B. thailandensis	
Campylobacter	Non-jejuni-coli group	
Edwardsiella	E. tarda, E. hoshinae, E. ictaluri	
Enterobacter	E. cloacae	
Neisseria	N. cinerea, N. meningitidis	
Pseudomonas	P. fluorescens, P. jessenii	
Streptococcus	S. mitis, S. oralis, S. pneumoniae	

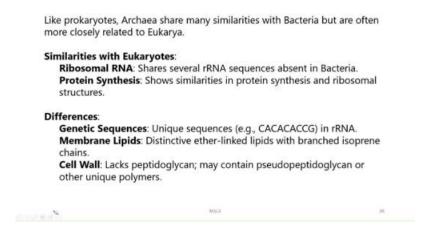
So, if you look at this figure, you can see that there is a difference in these linkages. While Archaea have ether linkages, Bacteria have ester linkages. The phospholipid bilayer in Bacteria and Eukarya is different from that of Archaea, as you can see in this diagram. Now, let us try to list these differences as mentioned in this table. So, if you take the cell membrane, as I have already told you, you see that Bacteria have ester linkages with unbranched fatty acids, while Archaea have ether linkages with branched isoprene chains.

In the cell wall, we have peptidoglycan in the case of bacteria, whereas archaea lack peptidoglycan and may contain pseudopeptidoglycan or other unique polymers. When it comes to the genome, bacterial genomes are small and less complex, whereas archaeal genomes are large and more complex. So, like prokaryotes, archaea share many similarities with bacteria but are often more closely related to eukarya. So, they are in between



the two: bacteria on one side and eukarya on the other side. So, what are the similarities with eukaryotes? The ribosomal RNA has several RNA sequences absent in bacteria. Then, when it comes to protein synthesis, it shows similarities in protein synthesis and ribosomal structures. Then come the differences.

Genetic sequences, unique sequences like this one—CACACACZ in RNA—are found. When it comes to the membrane lipids, we have distinct ether-linked lipids with branched isoprene chains, as we have already discussed in the figure. Then, in the table, we have mentioned that it lacks peptidoglycan and may contain pseudopeptidoglycan or other unique polymers. Let us see the evolutionary perspective of archaea. Archaea are considered among the most primitive life forms, resembling early cells from 4 billion years ago.



Modern archaea, these extremophiles, thrive in extreme environments similar to ancient conditions, such as hot, anaerobic habitats which are rich in sulfuric gases and salts. So how are Archaea classified? Classification in the Archaea domain is more challenging than

in bacteria due to the more recent exploration and discovery of Archaea species. Recent molecular studies have identified additional phyla and more complex classifications, reflecting the diversity and adaptability of Archaea. Archaeal organisms are widespread across diverse environments, with some thriving in extreme conditions such as cold or high temperatures, while others adapt to more moderate habitats.

Many archaea are extremophiles, thriving in extreme conditions such as high temperatures, high salt concentrations, or extreme pH levels. We have the archaea divided into various phyla, the major phyla, Euryarchaeota, which includes the methanogens, the halophiles, and several other diverse groups. Then we have Crenarchaeota, which includes thermophiles, acidophiles, and these are often found in hot and acidic environments. Let us discuss the Euryarchaeota first. Euryarchaeota is a diverse phylum containing extremophiles with various physiologies and shapes, including rods and cocci.

### Classification of Archaea

Classification in the Archaea domain is more challenging than in Bacteria due to the more recent exploration and discovery of archaeal species. Recent molecular studies have identified additional phyla and more complex classifications, reflecting the diversity and adaptability of Archaea.

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### Euryarchaeota

**Euryarchaeota** is a diverse phylum containing extremophiles with various physiologies and shapes, including rods and cocci.

These organisms can be gram-positive or gram-negative, depending on the presence of pseudomurein in their cell walls.

1. Methanogens are a notable group within Euryarchaeota, producing methane (CH<sub>a</sub>) from CO<sub>2</sub> and H<sub>2</sub>, and thriving in oxygen-free environments such as sediments, mud, and animal gastrointestinal tracts.

Methane production is crucial for their energy metabolism, and these species release over 2 billion tons of methane annually, with about one-third coming from cattle rumen.

These organisms can be gram-positive or gram-negative depending on the presence of pseudomurein in their cell walls. The first is the methanogens. These are notable groups

within Euryarchaeota, producing methane from carbon dioxide and hydrogen and thriving in oxygen-free environments such as sediments, mud, and animal gastrointestinal tracts. Methane production is crucial for their energy metabolism, and these species release over 2 million tons of

Methane annually, with about one-third coming from cattle rumen. For this reason, these species are also considered very important from the climate change point of view. Next come the halophiles. These are Archaea. They thrive in high-salt environments and hyperthermophiles, which survive at extremely high temperatures.

They require high salt concentrations, up to 36% NaCl, and oxygen for energy metabolism, thriving in highly saline environments like the Great Salt Lake, the Dead Sea, and salt evaporation ponds. Some species, known as halobacteria, use a red pigment to produce ATP in the presence of light, contributing to the vivid coloration of red herrings, the Red Sea, and salt ponds. Certain halophiles also thrive in extremely alkaline lakes with a pH above 11. Hyperthermophiles. These are archaea adapted to grow at very high temperatures, as the name suggests.

Halophiles are archaea which thrive in high-salt environments, and hyperthermophiles, which survive at extremely high temperatures.

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Certain halophiles also thrive in extremely alkaline lakes with a pH above 11.



Cluster of cells of Halobacterium sp. strain NRC-1

NASA, Public domain, via Wikimedia Common

They thrive at temperatures between 80 and 105 degrees and often tolerate high salt and acidity. They are typically found in volcanic waters, soils, and submarine vents. For instance, Thermoplasma flourishes in hot acidic environments, such as near coal mines, with a pH of 1 and temperatures around 60 degrees Celsius. Researchers have also discovered hyperthermophilic archaea in deep-sea sulfur vents, where they survive at temperatures up to 250 degrees Celsius and pressures of around 265 atmospheres. Crenarchaeota.

### 3. Hyperthermophiles are archaea adapted to grow at very high temperatures.

They thrive at temperatures between 80°C and 105°C and often tolerate high salt and acidity.

They are typically found in volcanic waters, soils, and submarine vents. For instance, Thermoplasma flourishes in hot, acidic environments, such as near coal mines with a pH of 1 and temperatures around 60°C.

Researchers have also discovered hyperthermophilic archaea in deep-sea sulfur vents, where they survive at temperatures up to 250°C and pressures of 265 atmospheres.



Crenarchaeota or Thermoproteota are primarily hyperthermophiles thriving at temperatures above 80 degrees centigrade. They are Gram-negative and exhibit diverse morphologies, including rods, cocci, filamentous, and irregular shapes. These archaea thrive in extreme environments such as hot sulfur springs with temperatures around 75 degrees centigrade, and acidic pH levels ranging from 2 to 3, as well as in volcanic vents. Additionally, some of these are found in cold deep-sea waters and polar regions, where temperatures can drop to as low as minus 3 degrees centigrade.

### Crenarchaeota

Crenarchaeota (or Thermoproteota) are primarily hyperthermophiles, thriving at temperatures above 80°C.

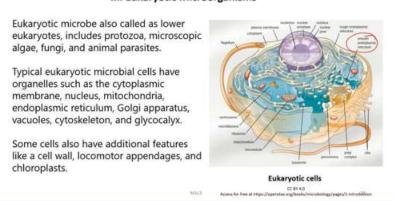
They are Gram-negative and exhibit diverse morphologies, including rods, cocci, filamentous, and irregular shapes.

These archaea thrive in extreme environments such as hot sulfur springs with temperatures around 75°C and acidic pH levels (2-3), as well as volcanic vents.

Additionally, some Crenarchaeota are found in cold deep-sea waters and polar seas, where temperatures can drop to -3°C.

Let us now move on to Section 3, which is a discussion on eukaryotic microorganisms. Here, we will be discussing fungi and protists, their characteristics, and their classification. Let us first define what eukaryotic microorganisms are. In this picture, you can see a eukaryotic cell, and you can see here a nucleus, which is enclosed by a clear nuclear envelope, unlike in prokaryotes. And then you have certain specialized structures like mitochondria here, and then you have a centrosome, and then you have smooth endoplasmic reticulum, Golgi complex, and you have lysosomes and peroxisomes. Now, eukaryotes may exist in microscopic form. So, these eukaryotic microbes are called lower eukaryotes, which include protozoa, then microscopic algae, fungi, and certain animal parasites. Typically, eukaryotic microbial cells have organelles, as I have already discussed, such as a cytoplasmic membrane, nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, cytoskeleton, and glycocalyx.

### III. Eukaryotic Microorganisms



Some cells also have additional features like cell walls, locomotor appendages, and chloroplasts. Fungi come under the eukaryotic microbes. The kingdom includes a wide range of organisms like bread molds, yeasts, powdery mildews, cup fungi, smuts, rusts, puffballs, and mushrooms. And these mushrooms are edible, while some are poisonous. We also use yeast for producing wine and bread, while there are other fungi which are actually involved in food spoilage.

Some fungi are microscopic, while others can grow to over two feet in size. Despite their differences, all fungi share several important properties. Number one, they are eukaryotic organisms characterized by complex cells with a nucleus, which we have discussed in the earlier slide. Their reproduction involves both sexual and asexual methods, resulting in spore production. Fungi exhibit growth in the form of hyphae.

These are filamentous structures that make up the mycelium or yeast, which are single-cell forms with hyphae typically showing apical growth, meaning they grow at the tips. Fungi are heterotrophic organisms, meaning they do not perform photosynthesis. They can be saprophytes, symbionts, or parasites, with some species parasitizing humans, animals, or plants. Fungi absorb nutrients through their cell membranes via osmotrophy, although a few possess the capability of phagotrophy, which involves ingesting solid food particles. To break down high molecular weight substrates, fungi secrete various extracellular enzymes.

### The Fungi

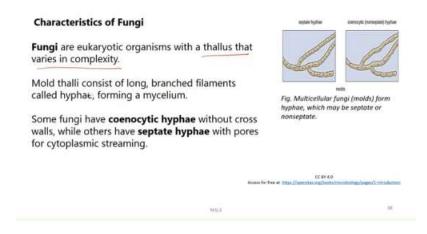
The kingdom *Fungi* includes a wide range of organisms, such as bread molds, yeasts, powdery mildews, cup fungi, smuts, rusts, puffballs, and mushrooms. Some fungi are microscopic, while others can grow to over two feet in diameter.

Despite their differences, all fungi share several important properties:

- · They are eukaryotic organisms, characterized by complex cells with a nucleus.
- Their reproduction involves both sexual and asexual methods, resulting in spore production.
- Fungi exhibit growth in the form of hyphae (filamentous structures that make up the mycelium) or yeasts (single-celled forms), with hyphae typically showing apical growth, meaning they grow at their tips.

Generally, fungi have sturdy polysaccharide-rich cell walls, primarily composed of chitin in most species, while some may contain mannans, galactans, or cellulose. What are the characteristics of fungi? So, you can see here one septate hypha and one syncytial or non-septate hyphae. So, here you can see certain divisions. These are the septate ones, and there are no such divisions over here in these non-septate fungi.

So, basically, fungi are aquatic organisms with a thallus, which has different kinds of complex forms. Most consist of long, branched filaments. These are called hyphae that form a mycelium. And as already discussed in this diagram, some fungi have syncytial hyphae without cross walls or septa, while others have septate hyphae with pores for cytoplasmic streaming. Cytoplasmic streaming.



If you take the case of yeast, they are unicellular fungi that reproduce by budding or spore formation and can form colonies, sometimes creating chains or pseudohyphae. These are not real hyphae. They appear like hyphae. Yeast cells are larger than bacteria, typically

spherical or egg-shaped, and contain eukaryotic organelles but lack flagella and cilia. Fungal classification.

Historically, fungal classification was based on structural differences and physiological or biochemical patterns. Today, DNA analysis and genome sequencing are used for understanding evolutionary relationships among various fungi. Fungi lacking a known sexual cycle are informally grouped as mitosporic fungi. These are often reclassified into a specific phylum once their sexual cycle is observed or when comparative genomics reveals a close relationship to other fungi. Fungi are generally categorized into five primary phyla based on their mode of sexual reproduction.

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For example, you have Chytridiomycota, which includes fungi with flagellated spores. Then you have Glomeromycota, which includes arbuscular mycorrhizal fungi forming symbiotic relationships with plant roots. Then you have Zygomycota, which are known for producing zygospores during sexual reproduction. And we have Ascomycota, known for asci, cyclic structures that contain spores. Then you have Basidiomycota, known for basidia, which are clustered structures where spores are produced.

### **Fungal Classification**

Fungi are generally categorized into five primary phyla based on their mode of sexual reproduction:

Chytridiomycota: Includes fungi with flagellated spores (zoospores).

Glomeromycota: Includes arbuscular mycorrhizal fungi forming symbiotic relationships with plant roots.

Zygomycota: Known for zygospores formed during sexual reproduction.

Ascomycota: Known for asci, sac-like structures containing spores.

Basidiomycota: Known for basidia, club-shaped structures where spores are

produced.

Let us discuss these in a little more detail. Chytrids are a diverse group with around 1,000 known species. They have chitin in their cell walls, which is a characteristic polysaccharide of fungi. These Chytridiomycota are unique among fungi. They have flagellated reproductive cells that allow them to swim.

They are mostly found in aquatic environments, both freshwater and marine. Many are saprophytic, such as Rhizophlyctis rosea. which decomposes cellulose in soil. Some chytrids also act as parasites on plants, insects, and amphibians. Glomeromycota.

### Chytridiomycota

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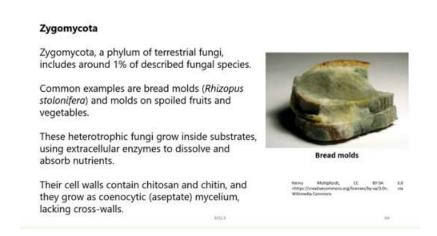
Many are saprophytic, such as Rhizophlyctis rosea, which decomposes cellulose in soils. Some chytrids also act as parasites on plants, insects, and amphibians.

These include arbuscular mycorrhizal fungi and are characterized by obligate symbiosis and asexual reproduction. Mycorrhizae denotes the close association between fungal mycelium and plant roots. Arbuscular mycorrhizal fungi create an extensive mycelial network in soil, up to 20,000 km per cubic meter, absorbing nutrients like phosphate and providing them to plants in exchange for carbohydrates. Over 80% of vascular land plants form mycorrhizal associations, benefiting from improved nutrient uptake and enhanced plant diversity. Arbuscular mycorrhizal fungi also help control pests and pathogens.

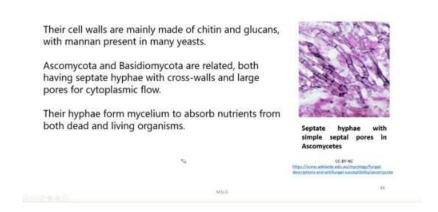
# Glomeromycota, including arbuscular mycorrhizal (AM) fungi, are characterized by obligate symbiosis and asexual reproduction. Mycorrhiza denotes the close association between fungal mycelium and plant roots. AM fungi create an extensive mycelial network in soil, up to 20,000 km per cubic meter, absorbing nutrients like phosphate and providing them to plants in exchange for carbohydrates. Over 80% of vascular land plant families form mycorrhizal associations, benefiting from improved nutrient uptake and enhanced plant biodiversity. AM fungi also help control pests and pathogens. Notable examples include: Acaulospora, Glomus, etc

Some notable examples include Entrophospora and Glomus. It is a phylum of terrestrial fungi, including around 1% of described fungal species. Common examples are bread molds, as you can see here in this picture, and molds on spoiled fruits and vegetables. So, basically, this is a harmful species responsible for food spoilage. These heterotrophic fungi grow inside substrates, using extracellular enzymes to dissolve and absorb nutrients.

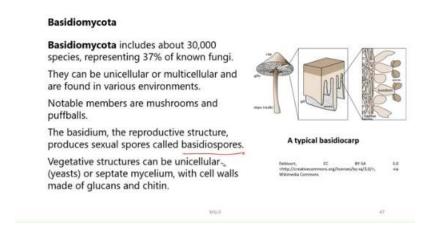
The cells contain chitosan and chitin, and they grow as syncytial mycelium lacking cross walls or septa. Ascomycetes, or sac fungi, represent about 70% of all known fungi and display considerable diversity. Some notable examples include Saccharomyces cerevisiae, which we now call the baker's yeast, then Morchella esculenta, then we have Penicillium chrysogenum and Neurospora crassa, which is a model organism like Saccharomyces cerevisiae. Ascomycetes have a vegetative structure that can be unicellular like yeasts or composed of septate filaments with multiple nuclei per segment.



So here we see the example of a septate hypha with simple septal pores in ascomycetes. The cell walls of ascomycetes are mainly made of chitin and glucans, with mannans present in many yeasts. Ascomycota and Basidiomycota are related, both having septate hyphae with cross walls and large pores for cytoplasmic flow. Their hyphae form mycelium to absorb nutrients from both dead and living organisms. The next is Basidiomycota, which includes about 30,000 species and represents around 37% of the known fungi.



They can be unicellular or multicellular and are found in various environments. Notable members are mushrooms and puffballs, and you can see here a typical basidiocarp with cap, gills, and stalk. The basidium, the reproductive structure, produces sexual spores called basidiospores. Basidiomycete structures can be unicellular or septate mycelium with cell walls made of glucans and chitin. Basidiomycota



Act as decomposers, form mycorrhizal relationships, and some are plant pathogens as well. Some examples include Cryptococcus neoformans, which causes lung infections. Then there is Agaricus campestris, which is an edible mushroom, and Amanita phalloides, which is deadly poisonous. So, you can see here basidiomycetes from Arndt-Steckel's 1904 work. Some beautiful diagrams over here of the particular species.

Next come the protists, which are mostly single-celled. But they perform complex functions akin to multicellular organisms. They thrive in aquatic environments, including water, damp soil, and mud, and may attach to surfaces or swim freely. The kingdom Protista, with over 64,000 species, is an artificial grouping due to its lack of common evolutionary traits. It includes unicellular eukaryotes and some multicellular forms, like slime molds.

### **Protists**

Protists are mostly single-celled but perform complex functions akin to multicellular organisms.

They thrive in aquatic environments, including water, damp soil, and mud, and may attach to surfaces or swim freely.

The kingdom Protista, with over 64,000 species, is an artificial grouping due to its lack of common evolutionary traits.

It includes unicellular eukaryotes and some multicellular forms like slime molds and brown algae.

Protists are divided into two main groups:

Subkingdom Algae and

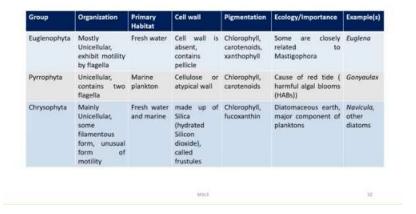
Subkingdom Protozoa.

And brown algae. Protists are divided into two main groups: the algae and protozoa. Algae are photosynthetic protists. They can be unicellular, colonial, or filamentous and vary widely in size, from a few micrometers to 100 meters. They possess chloroplasts and may contain additional pigments for yellow, red, and brown colors.

Most algae have a complex cell wall made of cellulose, contributing to the unique appearance of some types, like diatoms and dinoflagellates. So here you can see assorted diatoms, 2 microns to 200 microns, under light microscopy. Euglenids, such as Euglena, have a flexible pellicle composed of protein layers and microtubules. Algae, which often use flagella or gliding for movement, may have eyespots (stigma, as you can see here) to navigate toward light for photosynthesis. Microscopic algae are categorized into divisions based on chlorophyll types, pigments, cell coverings, stored food, and genetic factors.

## Algae are photosynthetic organisms that can be unicellular, colonial, or filamentous and vary widely in size, from a few micrometers to 100 meters. They possess chloroplasts and may contain additional pigments for yellow, red, and brown colors. Most algae have a complex cell wall made of cellulose, contributing to the unique appearance of some types, like diatoms and dinoflagellates. Assorted diatoms (2 µm to 200 µm) under light microscopy of some types, like diatoms and dinoflagellates.

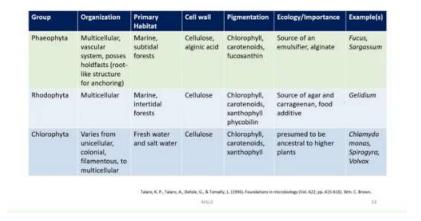
So they may be classified as Euglenophyta, Pyrophyta, Chrysophyta, Pheophyta, Rhodophyta and Chlorophyta. Euglenophyta are mostly unicellular and exhibit motility by flagella, found in freshwater. They do not have a cell wall, contain a pellicle, and have pigments like chlorophyll, carotenoids, and xanthophyll. Some are closely related to Mastigophora, and examples include Euglena.



Then we have Pyrophyta, which are also unicellular and contain two flagella. They live in marine environments and have cellulose or an atypical wall. They contain chlorophyll and carotenoids as pigments, and the cause of red tide or harmful algal blooms is ascribed to Pyrophyta. Then we have Chrysophyta. These are mainly unicellular, some filamentous in appearance, with an unusual form of motility.

They are found in both freshwater and marine water, made up of silica, hydrated silicon dioxide called frustules, found in their cellula. They have pigments like chlorophyll and fucozentine. Mostly, they are the diatomaceous art and major component of the planktons.

Examples include navicula. Then we have pheophyta, which are multicellular vascular systems, possess hole-fast or root-like structure for anchoring.



Primarily, they are found in marine subtidal forests. Their cell wall contains cellulose and alginic acid and have chlorophyll, carotenoids and fucosanthin. And they serve as source of emulsifiers and famous for providing alginate. Examples include fucus sargassum. And then we have rhodophyta.

They are multicellular, found in marine intertidal forests. Cellules include cellulose, have chlorophyll, carotenoids, gentophyll, and phycobilin. They are source of agar and carrageenan and other food additives. Examples include gelidium. Chlorophyta have various forms ranging from unicellular, corneal, filamentous to multicellular.

They are found in freshwater as well as in saltwater, have cellulose in their cell walls, and apart from chlorophyll and carotenoids, they have xanthophyll as pigments. These are presumed to be ancestral to higher plants. Examples include Chlamydomonas, Spirogyra, and Bulwoks. Protozoa. Protozoa are a subgroup within the kingdom Protista, comprising approximately 65,000 species of single-celled, animal-like microorganisms.

### Protozoa

Protozoa are a subgroup within the Kingdom Protista, comprising approximately 65,000 species of single-celled, animal-like microorganisms.

Characteristics: They are known for their motility and varied forms of movement, such as using cilia, flagella, or pseudopodia.

Reproduction: Pathogenic protozoa often reproduce asexually via simple cell division of the trophozoite (the active feeding stage).

Many form cysts with a protective outer layer to survive outside a host, which can later release trophozoites when conditions are favorable.

Life Cycles: Protozoa can have complex life cycles, including both asexual and sexual phases, particularly in groups like apicomplexans.



protozoans

Clockwise from top left: Blephenisma japonicum, a ciliate; Glardie muris, a parasitic flagelline: Centropyxis ocuieeta, a testate (shelled) amoeba; Peridinium willei, a dinoflagellate; Chaos carolinense, a raked amoebosoan; Desmerella monififormis, a chaomiliani.

Respectively: Frank fiss, Sergey Karpov, CDCP Sir Stan Erlandson, Pictur-eposit, Theory Amet, dr. Zuykit Huigi, CC SY: SA 4.E, via Wikimedia Communic

Characteristics. They are known for their motility and varied forms of movement, such as using cilia, flagella, or pseudopodia. Reproduction: Pathogenic protozoa often reproduce asexually via simple cell division of the trophozoite, the active feeding stage. Many form cysts with a protective outer layer to survive outside a host. These can later release trophozoites when conditions are favorable.

Protozoa can have complex life cycles, including both asexual and sexual phases, particularly in groups like Apicomplexa. Here, you can see various protozoans. Clockwise, from top left, you can see Blepharisma japonicum, which is a ciliate. Then, Giardia muris, a parasitic flagellate. Then, you have Centropyxis, Centropyxis aculeata, a testate or shelled amoeba.

Then, we have Peridinium willi, a dinoflagellate. Then, we have Karos carilensins, a naked amoeba zone. Classification of protozoa. There are various ways to classify protozoa. Let us discuss them one by one.

The first is based on motility, reproduction, and life cycle characteristics. We have Mastigophora. These are flagellated protozoa, some with amoeboid motility, reproduce sexually, and have both cyst and trophozoite stages. Some examples include Giardia, Leishmania, and Trichomonas. Then we have Sarcodina.

### Classification of Protozoa

A. Based on motility, reproduction, and life cycle characteristics

### 1. Mastigophora:

Flagellated protozoa, some with amoeboid motility; reproduce sexually and have both cyst and trophozoite stages.

Examples: Giardia, Leishmania, Trichomonas.

### 2. Sarcodina:

Amoeboid protozoa that reproduce asexually through fission; mostly free-living.

Examples: Arcella, Amoeba proteus, Foraminifera.

These are amoeboid protozoa that reproduce asexually through fission, mostly free-living. Examples include Arcella, Amoeba proteus, and Foraminifera. Then we have Ciliophora, which is characterized by cilia, exists as trophozoites and cysts, mostly free-living and harmless. Some examples include Paramecium, Blepharisma, and Balantidium coli. Then comes the Apicomplexa.

These are non-mortal except for the male gametes. They reproduce sexually and asexually with complex life cycles. All of them are parasitic. We have famous examples like Plasmodium, Toxoplasma gondii, and Cryptosporidium under Apicomplexa. Another classification of this group is based on genetic analysis and genomics.

### 3. Ciliophora:

Characterized by cilia; exist as trophozoites and cysts; mostly freeliving and harmless.

Examples: Paramecium, Blepharisma, Balantidium coli.

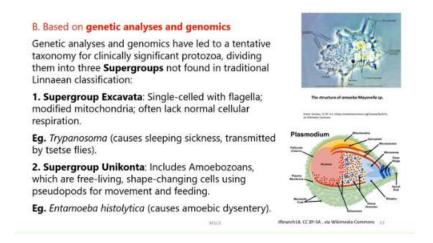
### 4. Apicomplexa:

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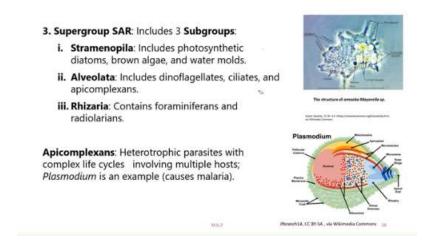
Genetic analysis and genomics have led to a tentative taxonomy for clinically significant protozoa, dividing them into three supergroups not found in traditional linear classification. The first supergroup is Excavata. These are single cells with flagella, modified mitochondria, and often lack normal cellular respiration. For example, we have

Trypanosoma, which causes sleeping sickness, and these are transmitted by tsetse flies. The next supergroup is called Unikonta.



These include amoebic organisms, which are free-living, shape-changing cells using pseudopods for movement and feeding. We have members like Entamoeba histolytica, which causes amoebic dysentery. The third supergroup is called SAR, and it includes three subgroups. SAR comes from the first letter of the three subgroups and is therefore an acronym. The first member of this subgroup is Stramenopiles.

This includes photosynthetic diatoms, brown algae, and water molds. Then you have alveolata, which includes dinoflagellates, ciliates, and apicomplexans. Then we have rhizaria, which contains foraminiferans and radiolarians. Apicomplexans are heterotrophic parasites with complex life cycles involving multiple hosts. For example, Plasmodium is one which causes malaria.



So, with this, we come to the end of this lecture, and then we will discuss the various methods and tools used in classification in the next lecture. Thank you. Amen.