Introduction to microbiology

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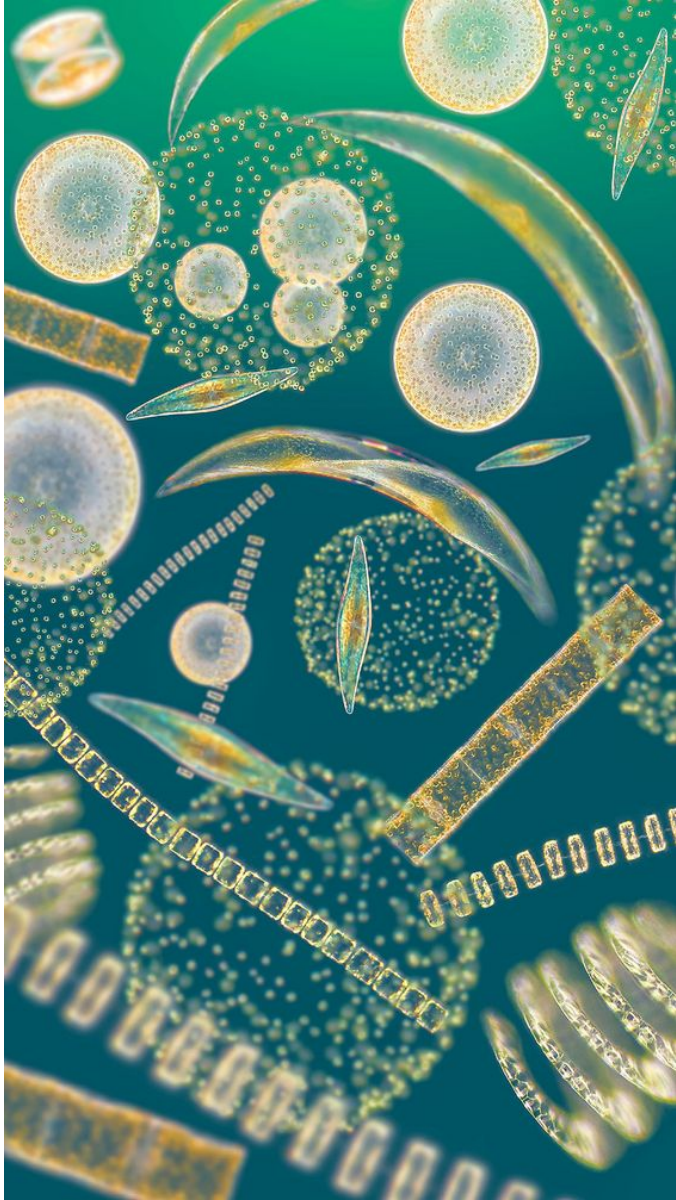
Outline

1. Terminology and classification
2. Practical exercise
3. Microbial communities and advanced methods of identification



Microbiology

- study of all living organisms that are too small to be visible with the naked eye (microorganisms)
- microorganisms are spread in water, soil, air, bodies of animals, in plants

Viruses

Algae

Fungi

Metazoa

Protozoa

Bacteria & Archaea

BartCo/iStock

Microbiology timeline

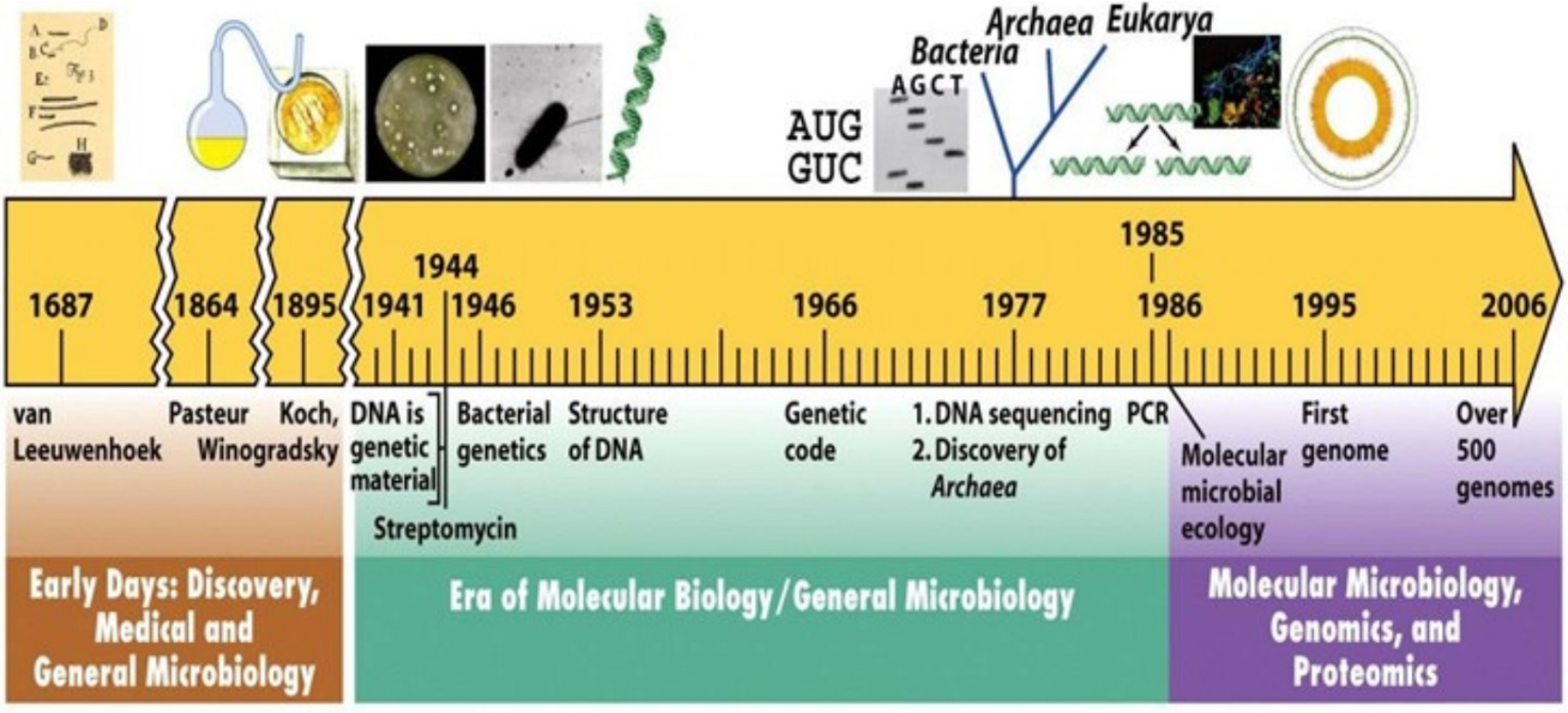
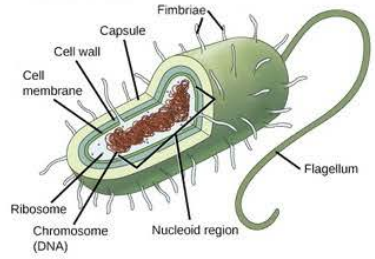


Figure 1-17 Brock Biology of Microorganisms 11/e

Eukaryotic vs Prokaryotic Cells



Prokaryotic

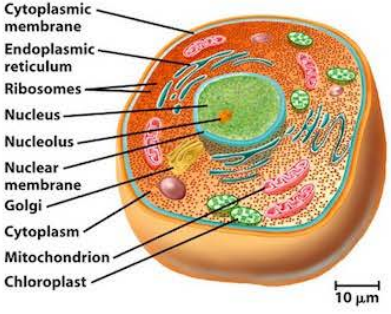
Prokaryotic cells are found in bacteria. They are less complex than Eukaryotic cells.

No Nucleus

Prokaryotic cells do not have a nucleus. They also do not have any membrane bound organelles.

DNA of Prokaryotic cells

Prokaryotic cells have smaller, circular DNA. DNA for prokaryotic cells is held in a nucleoid.



Eukaryotic

Eukaryotic cells are found in humans. They are also found in plants, animals, fungi, and insects.

Nucleus

Eukaryotic cells have nuclei and other membrane bound organelles.

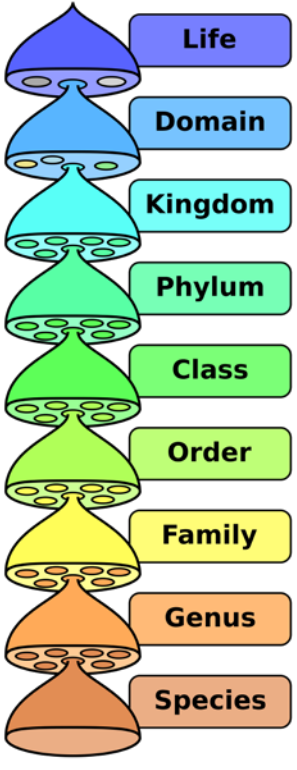
DNA of Eukaryotic cells

Eukaryotic cells have linear strands of DNA.

"Eukaryotic and Prokaryotic Cells: Similarities and Differences." Study.com. N.p., n.d. Web. 05 Jan. 2017.

https://www.youtube.com/watch?v=zZtcMBTQaS4&index=3&list=PLTH8ahUIcvwRCscNWDRcD2ZrzBjbrPLt9

Classification of living organisms



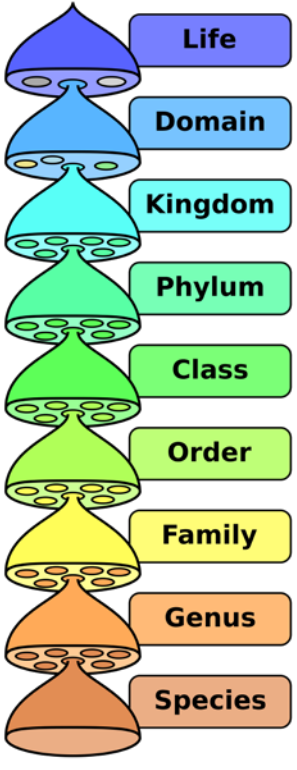
Taxonomic classification

- a hierarchical system for classifying organisms
- arranging organisms into groups based on similarities of structure, origin, etc
- all organisms are classified into 3 domains: archaea, eubacteria and eukaryotes (viruses are not classified as living organisms)
- the principal taxa for classification are kingdom, phylum, class, order, family, genus, species
- Species are named according to **Binomial Nomenclature**

two name naming system

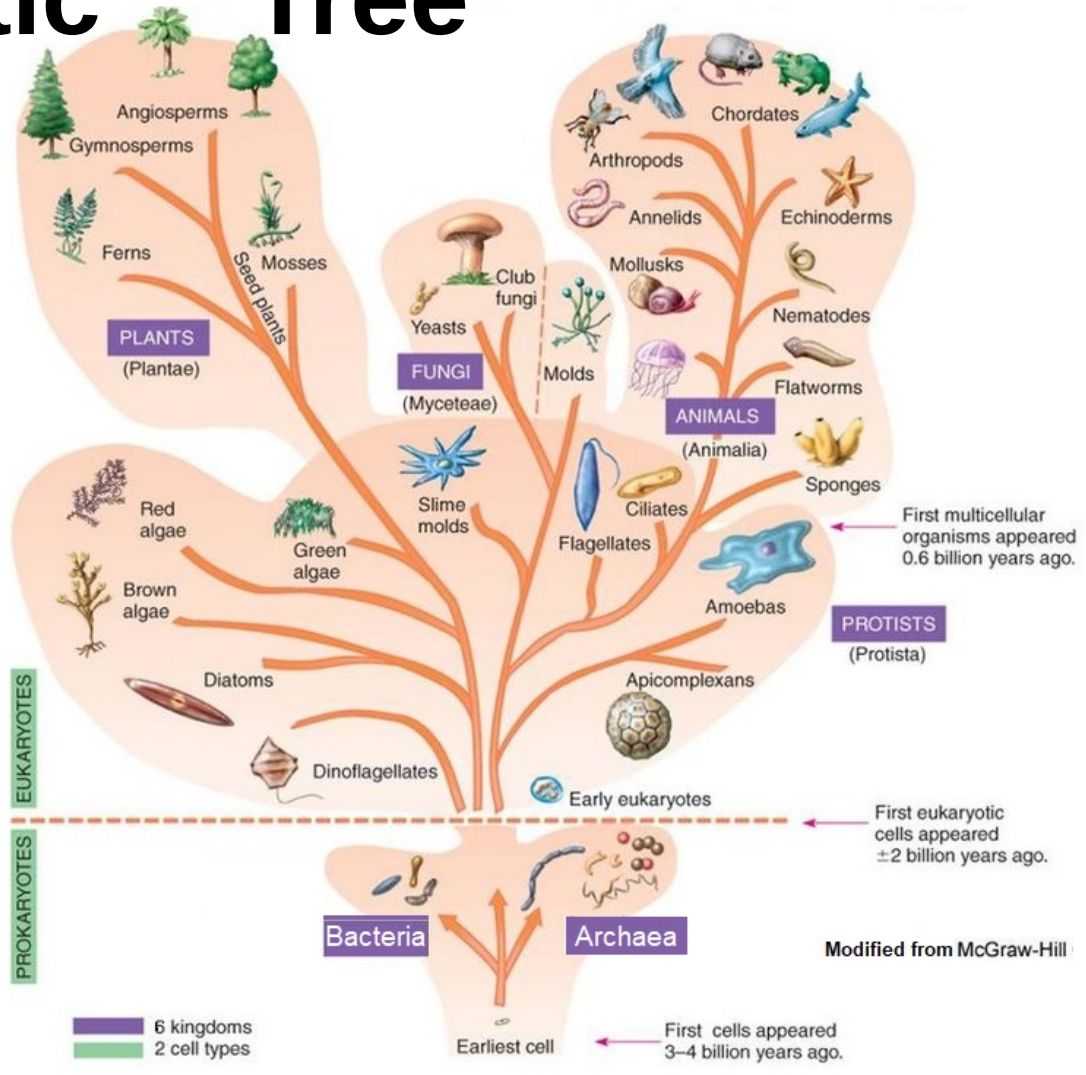
  - First name is the Genus, capital
  - Second name is the species, lower case
  - Both are italicized
  - Example: *Escherichia coli,* or *E.coli*
  - Strains; minor differences with in species:
    - *E. coli* strain B or *E.coli* strain K-12

Classification of living organisms

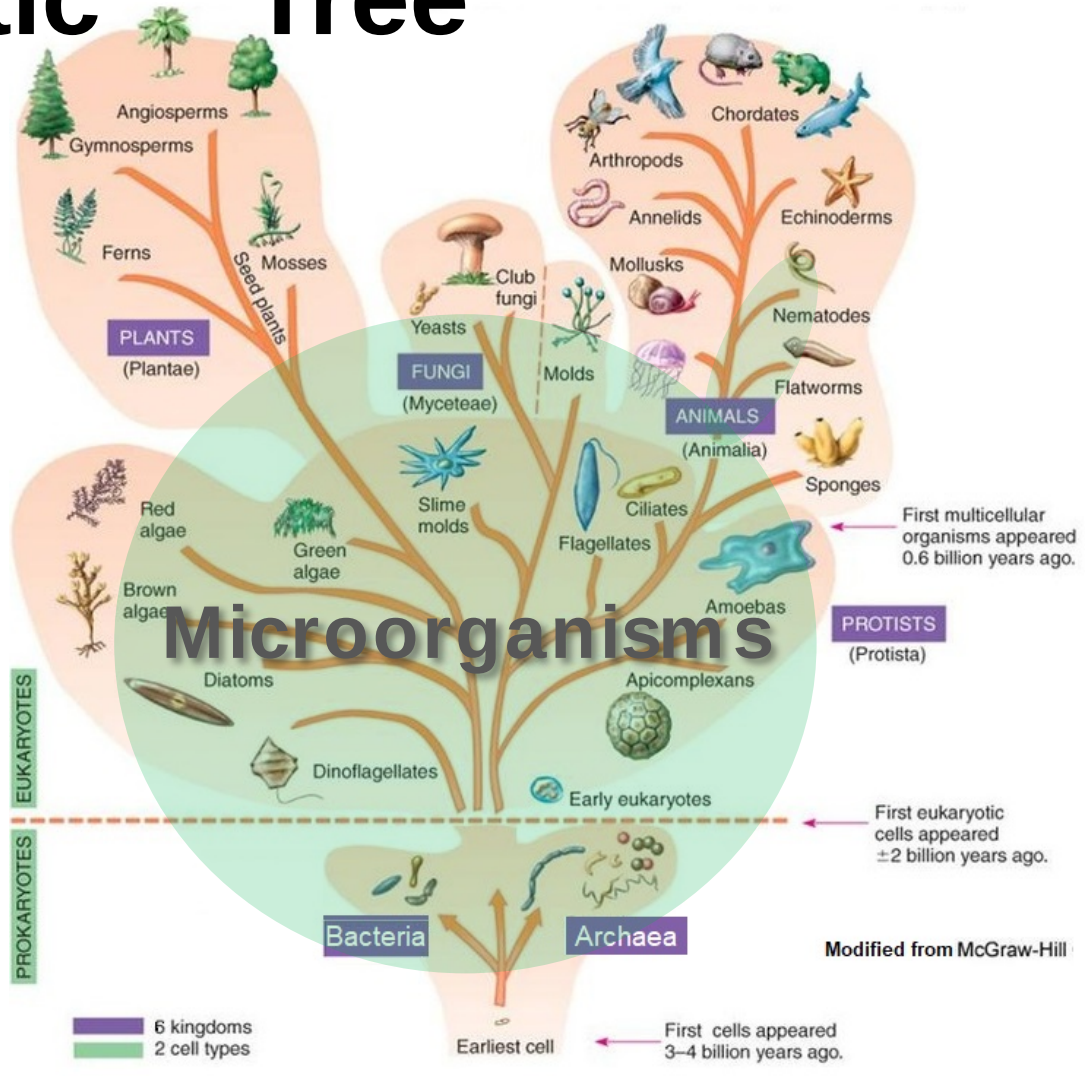


Domain	Bacteria	Archaea	Eukarya			
Kingdom	**Eubacteria**	**Archaebacteria**	**Protista**	**Fungi**	**Plantae**	**Animalia**
Cell Type	Prokaryote	Prokaryote	Eukaryote	Eukaryote	Eukaryote	Eukaryote
Cell Structures	Cell walls with peptidoglycan	Cell walls without peptidoglycan	Cells walls of cellulose; some have chloroplasts	Cell walls of chitin	Cell walls of cellulose; chloroplasts	No cell walls or chloroplasts
Number of Cells	Unicellular	Unicellular	Most unicellular; some colonial; some multicellular	Most multicellular; some unicellular	Multicellular	Multicellular
Mode of Nutrition	Autotroph or Heterotroph	Autotroph or Heterotroph	Autotroph or Heterotroph	Heterotroph	Autotroph	Heterotroph
Examples	Streptococcus and Escherichia coli	Methanogens and Halophiles	Amoeba, Paramecium, Giant Kelp	Mushrooms and Yeast	Mosses, Ferns, and Flowering Plants	Sponges, Worms, Insects, Fish, and Mammals

Phylogenetic Tree



Phylogenetic Tree



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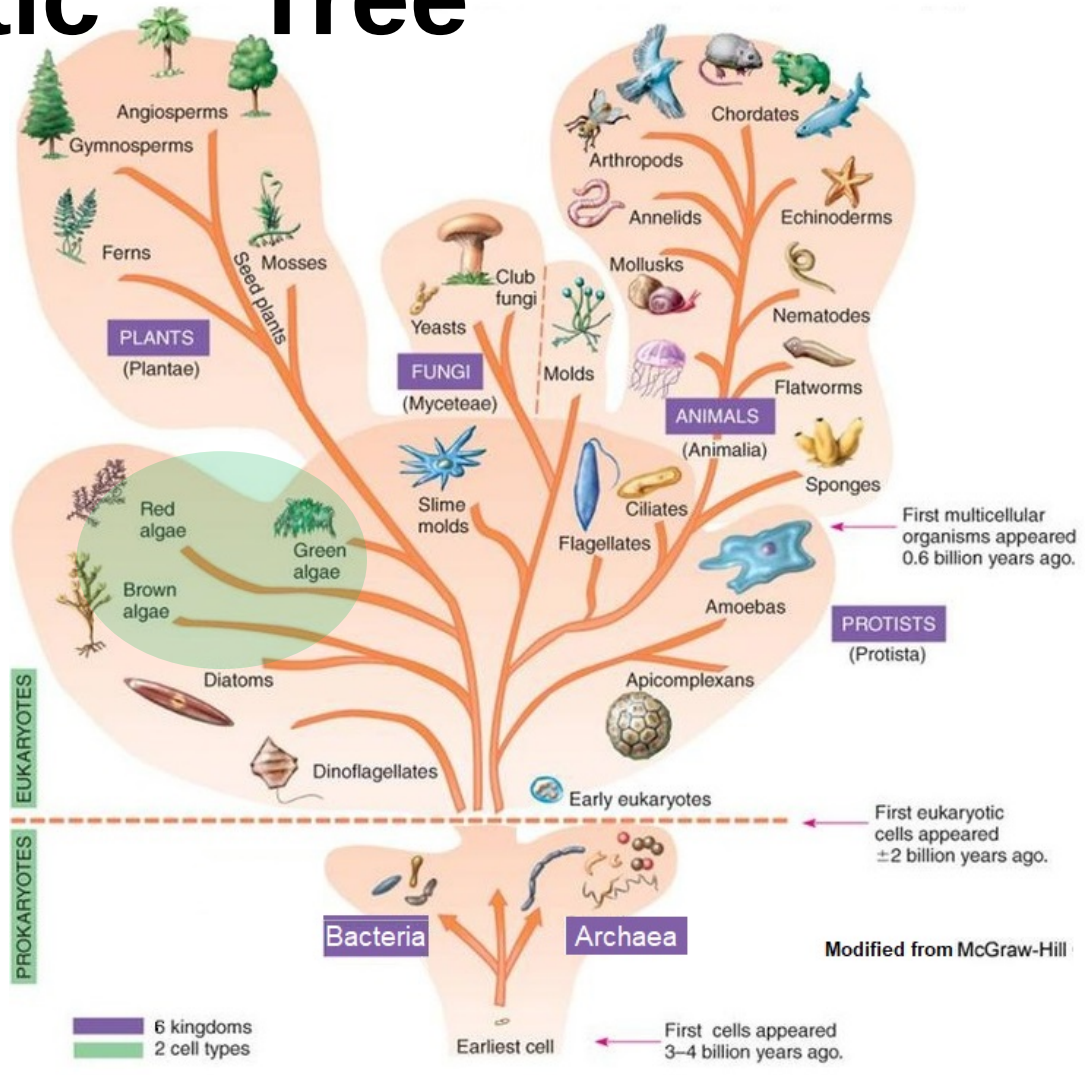
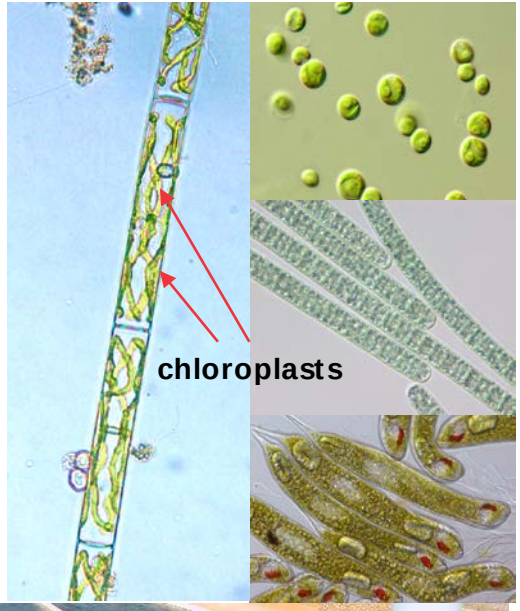


Algae

- photosynthetic aquatic eukaryotes
- unicellular or filamentous
- produce oxygen and consume carbon dioxide, act as the base for the aquatic food chain, remove nutrients and pollutants from water, and stabilize sediments

- can be the reason of activated sludge bulking
- used as fertilizers, for algae based wastewater treatment, bio-fuel production



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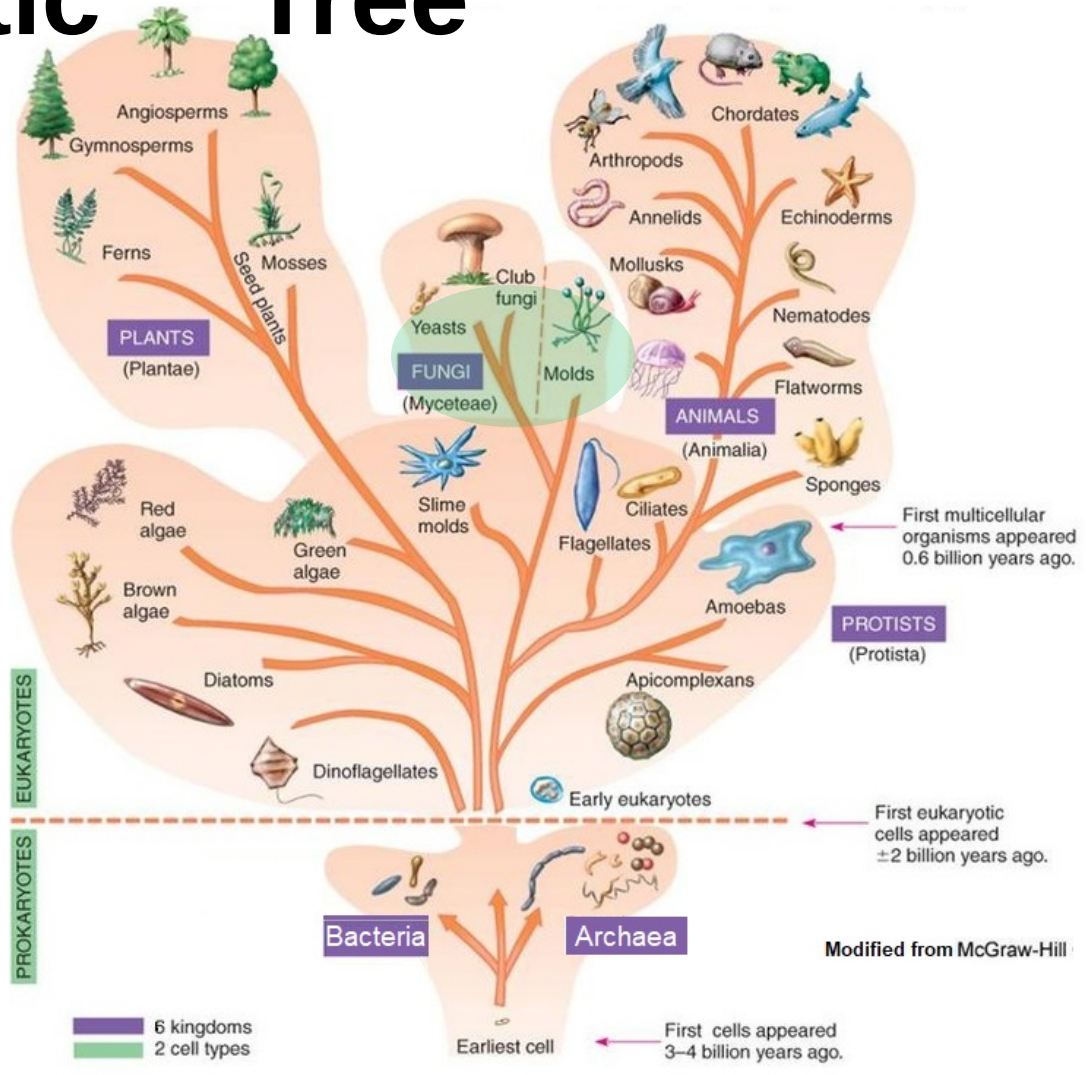
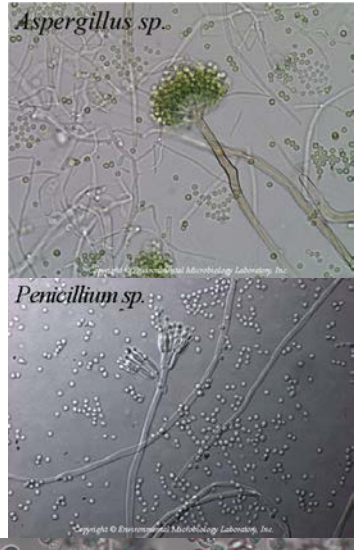
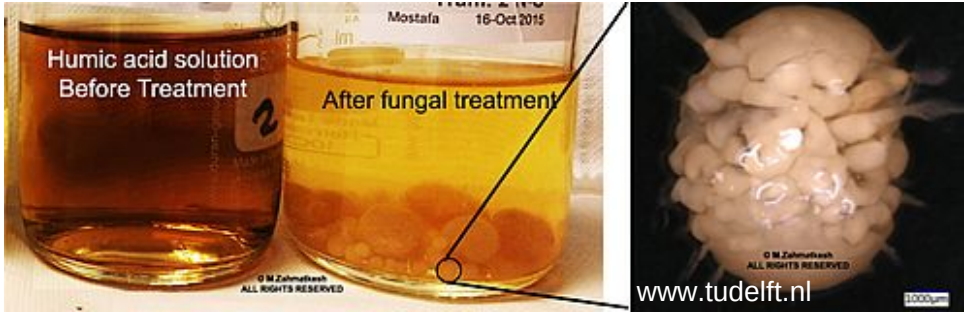
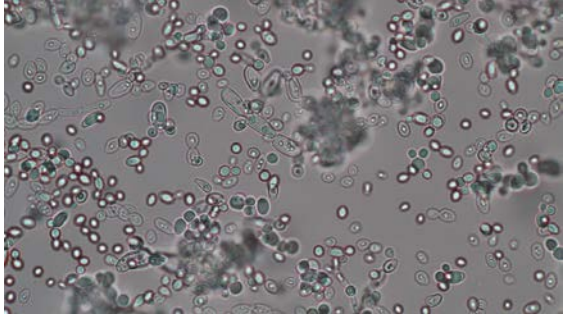


Fungi

- Eukaryotic (closer to Animals than to Plants)
- >1 000 000 species, mostly multicellular (except unicellular yeasts)
- various habitats: water (including sea water), soil, air, bodies of animals
- use organic compounds as a source of carbon and energy
- produce variety of enzymes (extracellular digestion)
- used in agriculture, medicine, environmental biology, biotechnology







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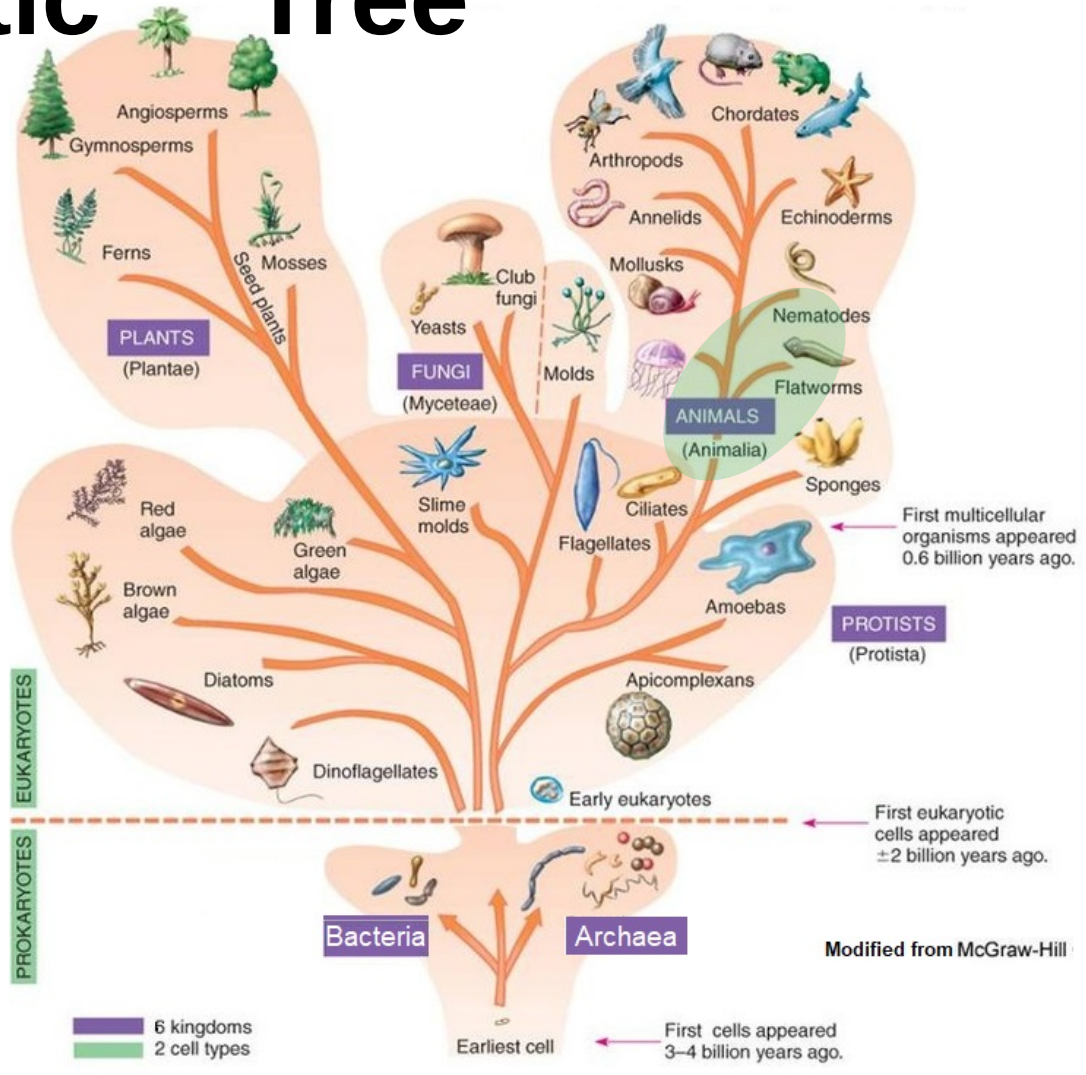
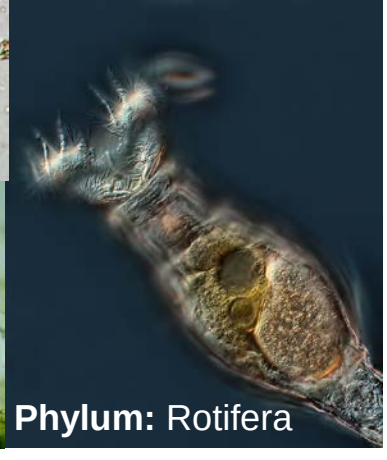
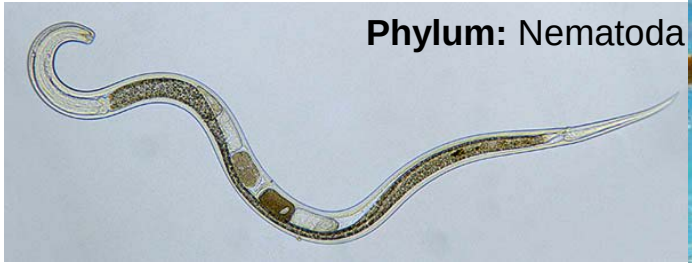


Metazoa

- multicellular eukaryotes (Animalia)
- aerobic conditions
- feed on bacteria, fungi, protists
- bioindicators of water treatment efficiency





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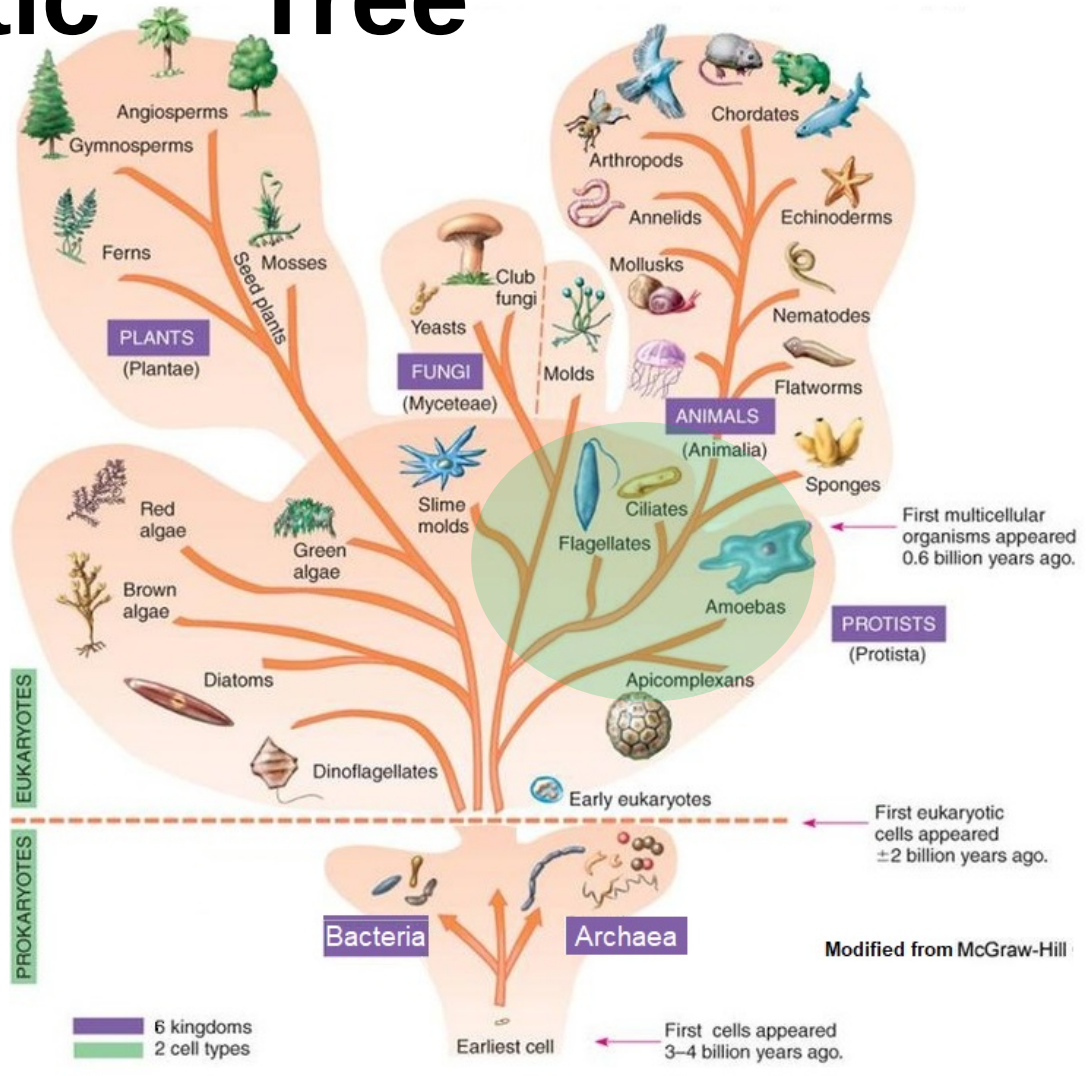
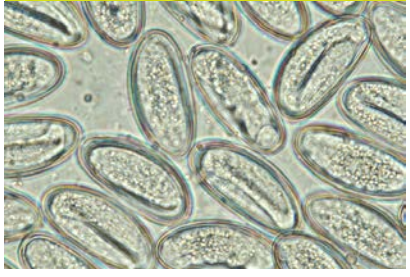


Protozoa

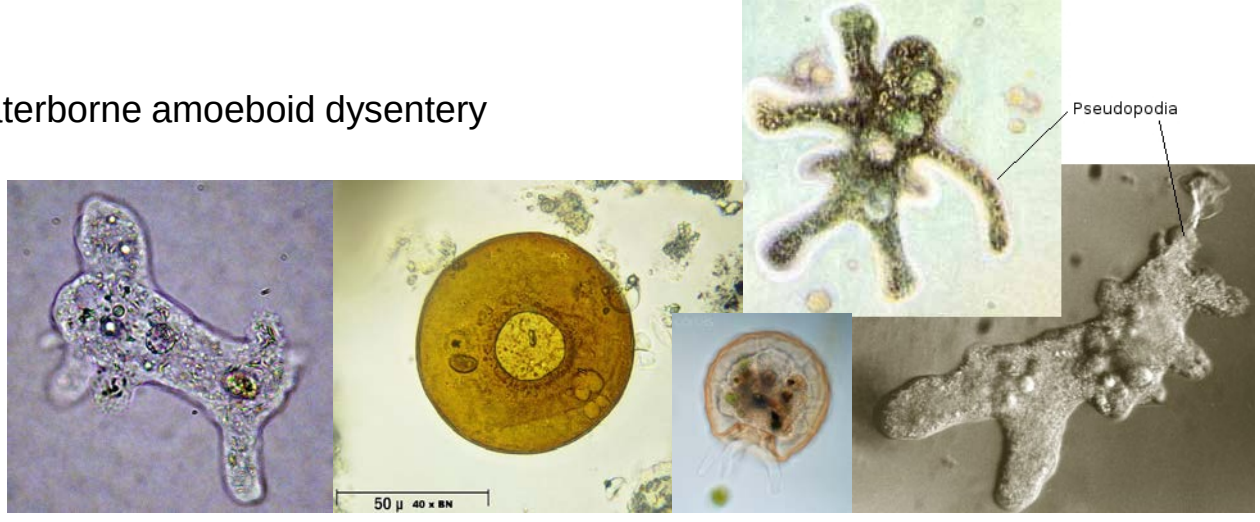
- animal-like Protists
- unicellular eukaryotes
- motile
- free-living (feed on bacteria and other microorganisms) or parasitic
- classified on their means of motility
- bioindicators of water treatment efficiency



Enterobius vermicularis eggs

Sarcodina

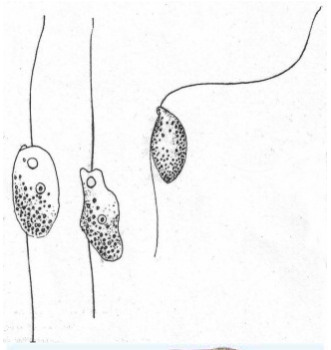
- the largest protozoan phylum:11,500 living species and 33,000 fossil species
- move by cytoplasmic streaming in pseudopods (false “feet”)
- In activated sludge, indicate start-up conditions or recovery from toxicity, washout, and organic overload
- *Entamoeba histolytica* – waterborne amoeboid dysentery

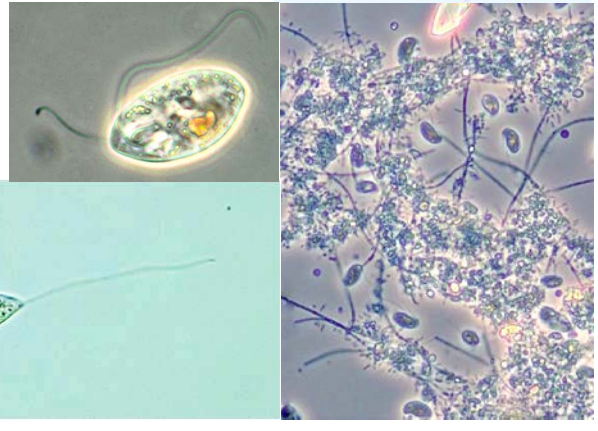


Mastigophora (*Flagellates*)

- one or more flagella (hair-like structure)
- free-living species are indicators of young activated sludge
- Several dangerous parasites

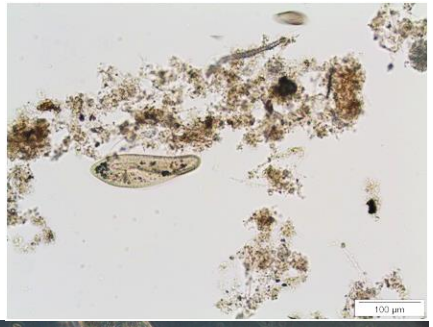
  Giardia lamblia is an important contaminant of drinking water, resistant to the disinfectant action of chlorine

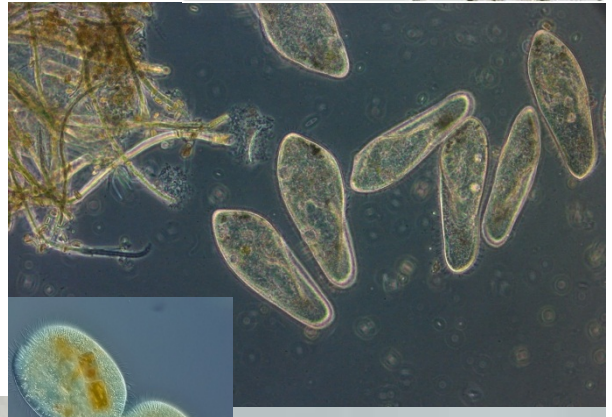


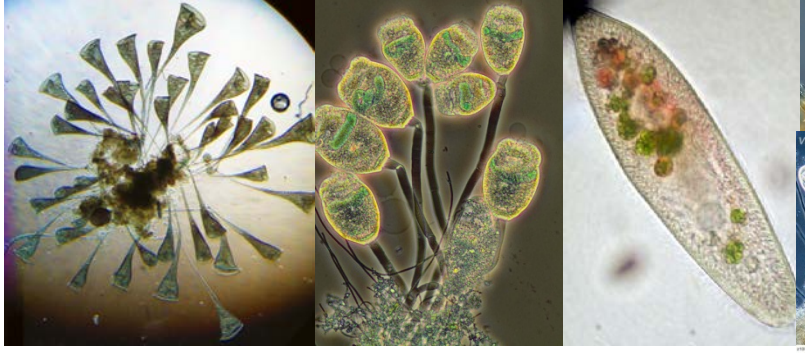


Ciliophora *(Ciliates)*

- phylum Ciliophora include ~8000 species
- move by cilia (multiple shirt hair-like structures)
- feeding mechanisms involve a mouth and cilia
- dominant in the presence of mature flocs and low BOD in the mixed liquor

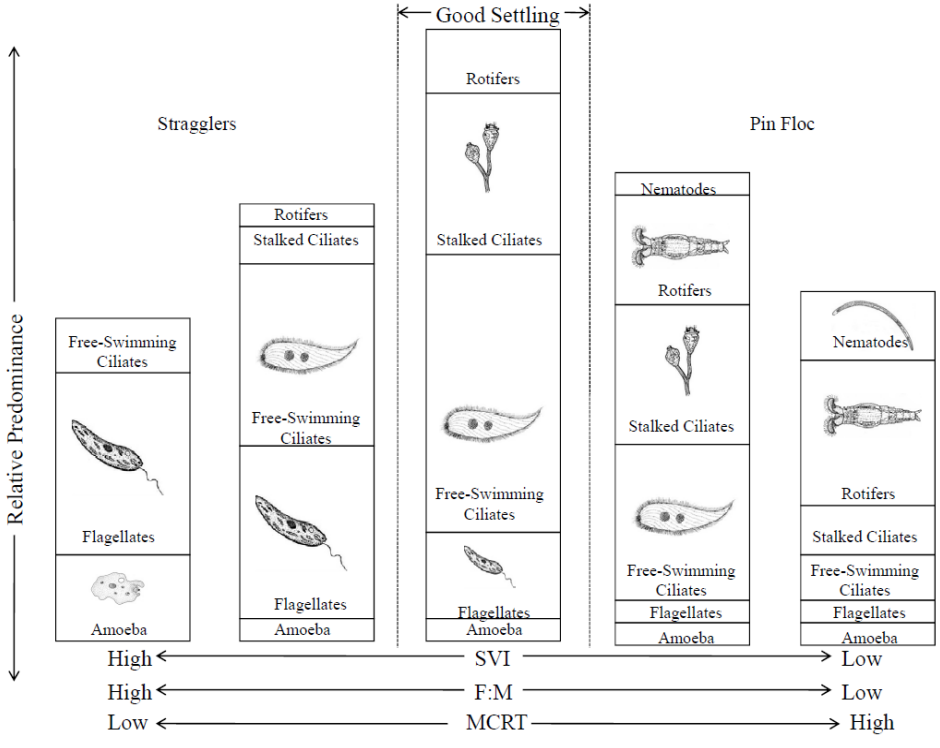


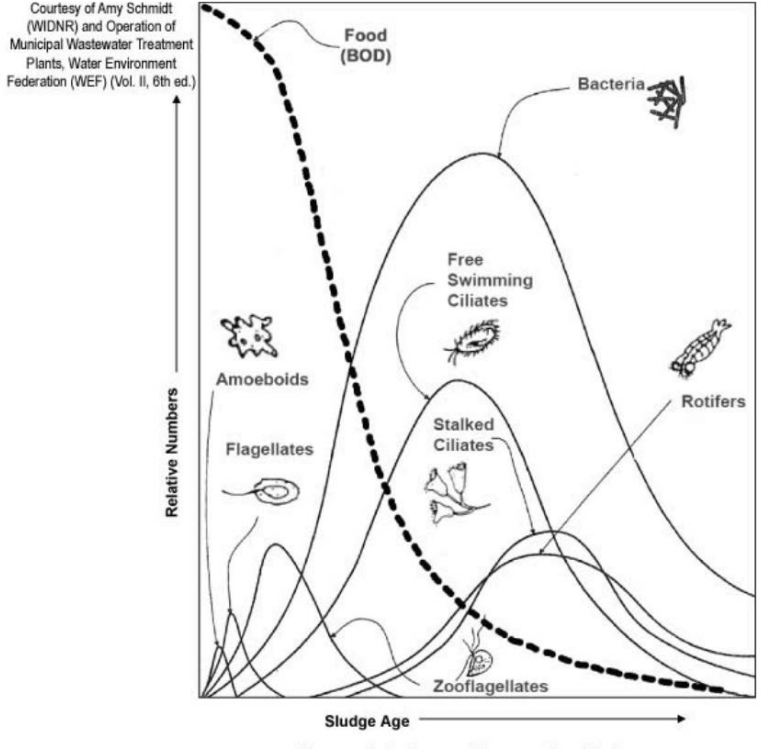




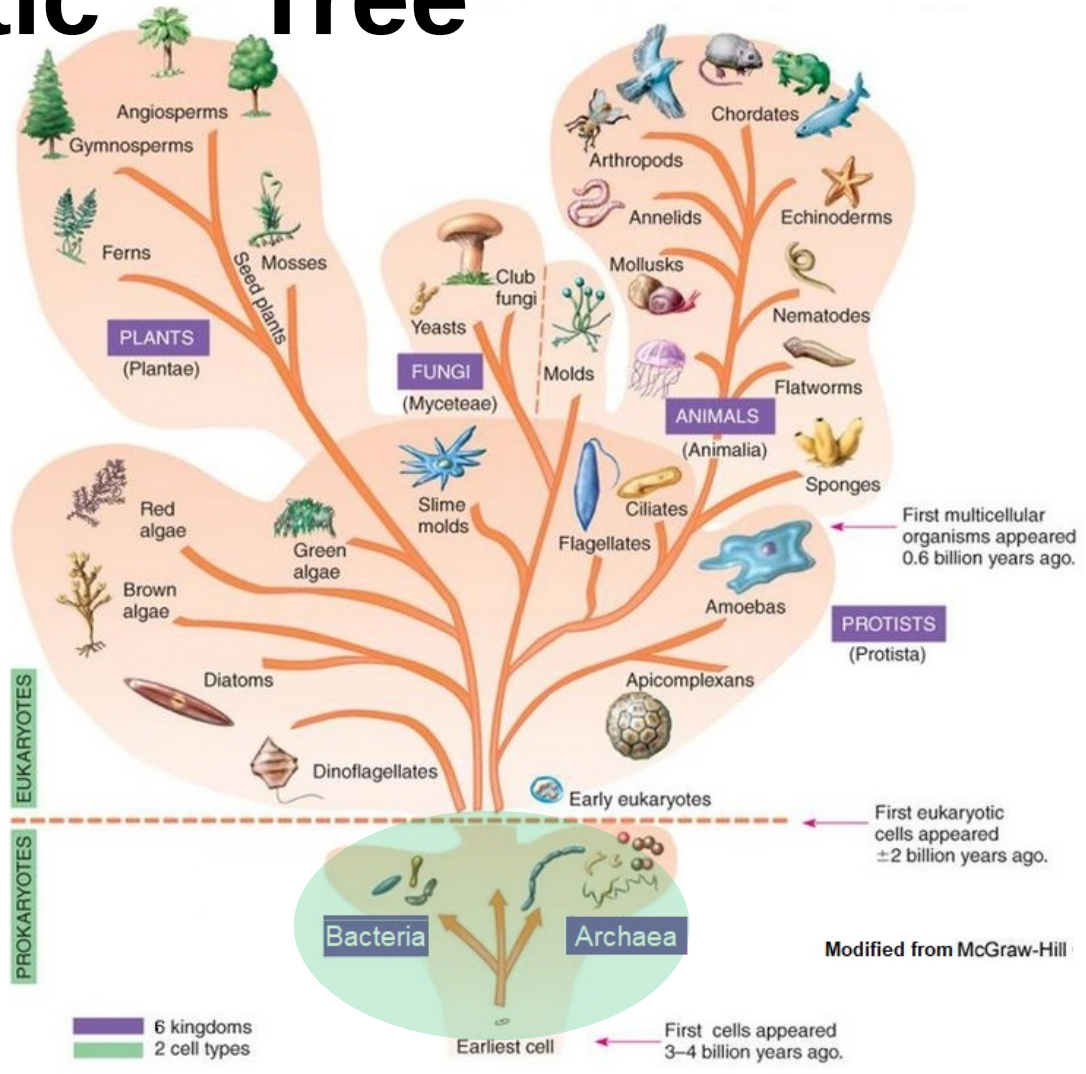
Size comparison

Wastewater treatment bioindicators



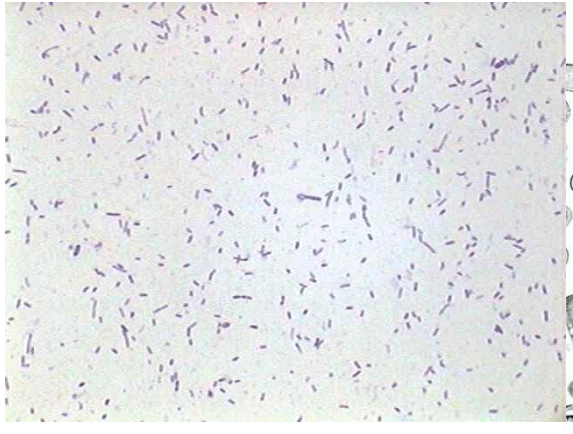


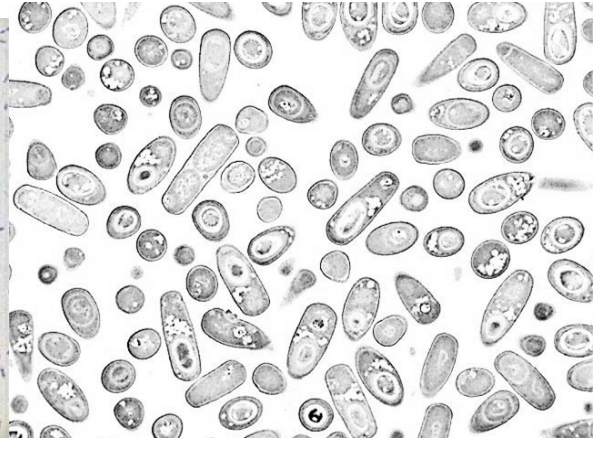
Phylogenetic Tree



Bacteria and Archaea

- simplest celled organisms with lack of a membrane-enclosed nucleus
- size - 0.02–400 μm (mostly 2-8).
- remove organic materials by microbial respiration and synthesis





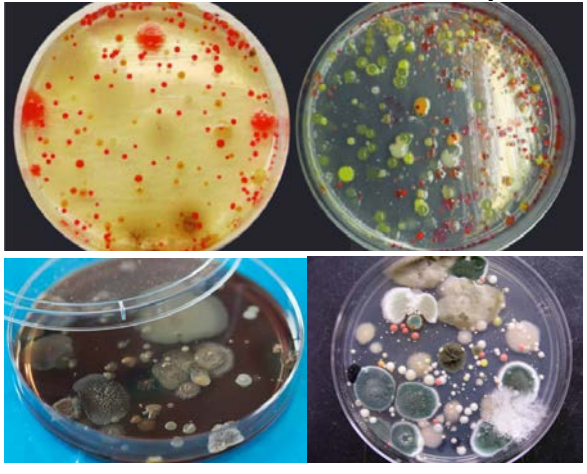
Identification of bacteria

- bacteria are generally grouped into species according to their morphological, physical and metabolic characteristics
- the most typically identification starts with morphological observation, the colony appearance and the gram staining and then individual features tested

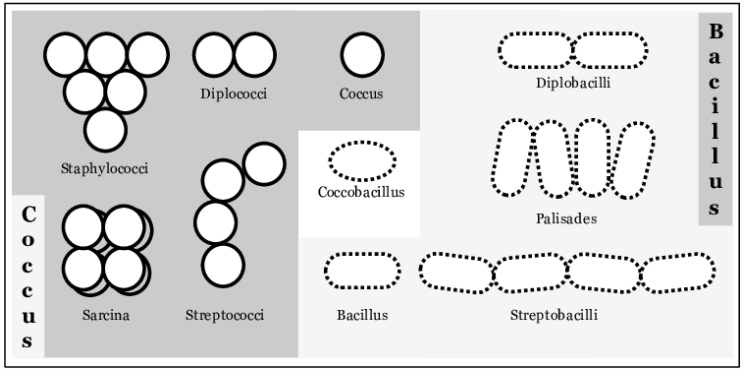
A *colony* is a visible mass of microorganisms on solid media all originating from a single mother cell

***Microbiological culture* is a method of multiplying microorganisms on selective media in laboratory conditions**

Colonies on Petri dishes/ culture plates

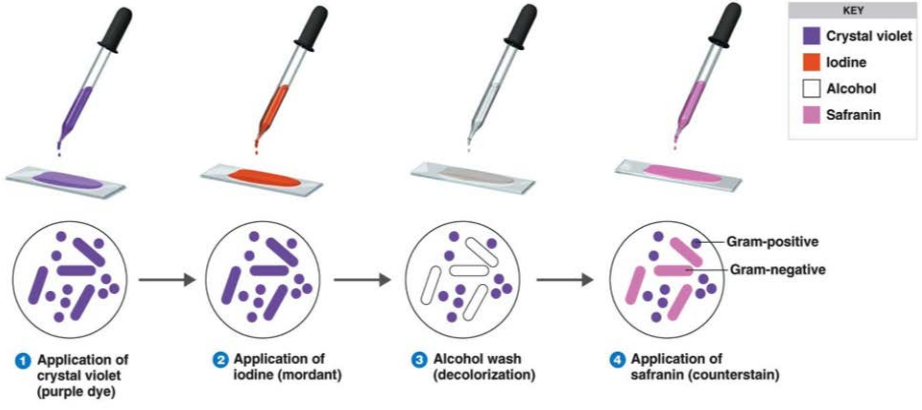


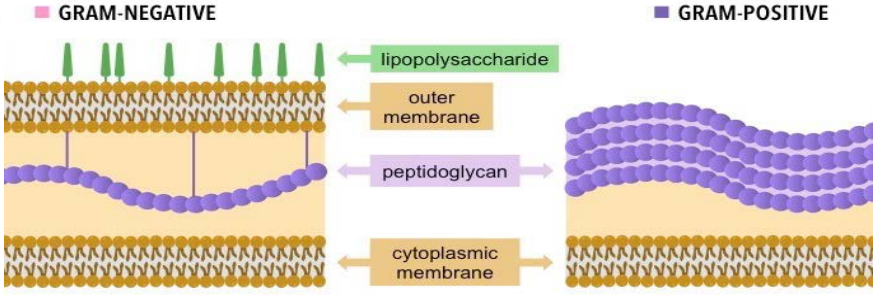
Shapes and arrangements



Identification of bacteria

- cell wall structure

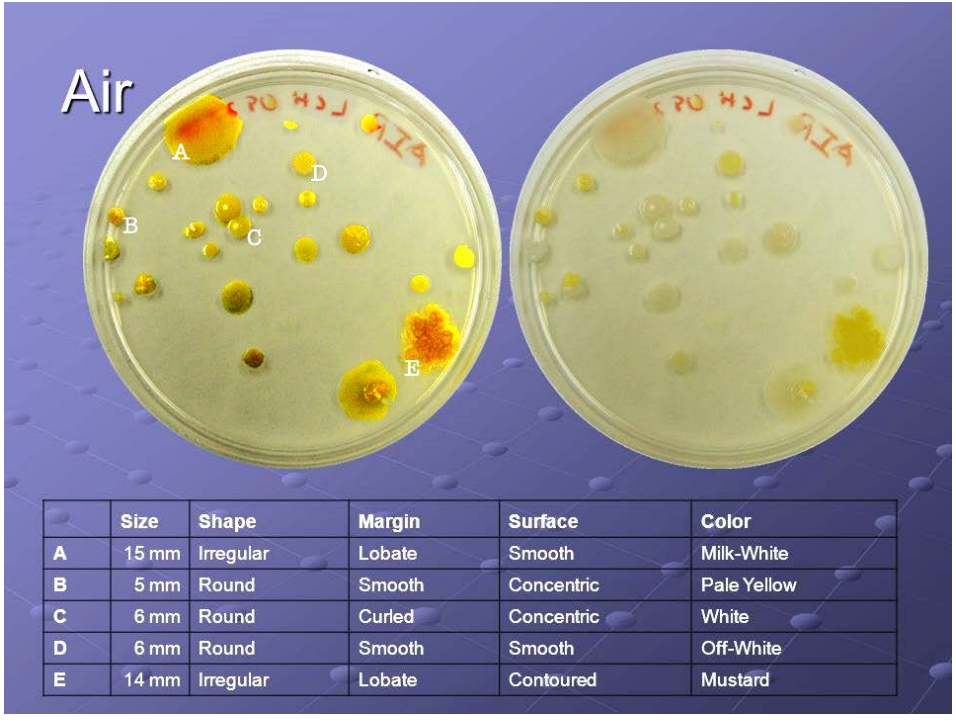




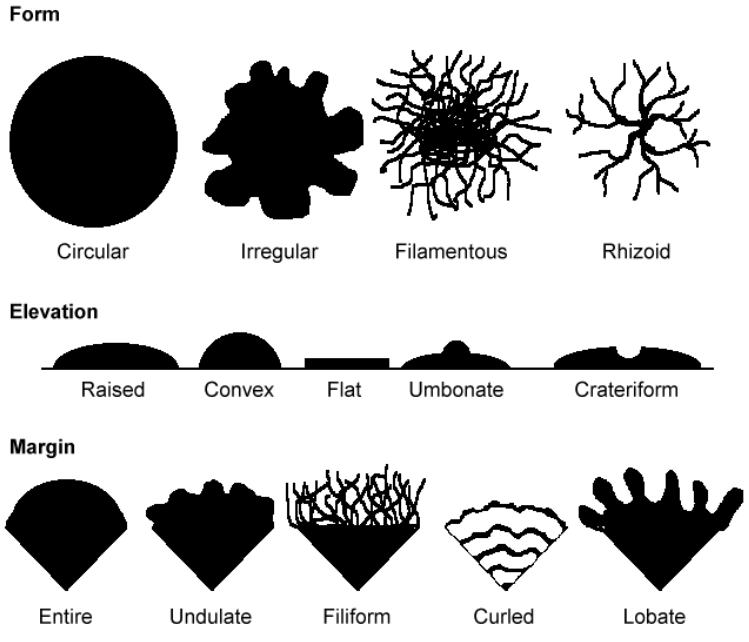
- about sixty more tests

http://www.microrao.com/identify.htm

The Examination of Bacterial Colonies



	Size	Shape	Margin	Surface	Color
A	15 mm	Irregular	Lobate	Smooth	Milk-White
B	5 mm	Round	Smooth	Concentric	Pale Yellow
C	6 mm	Round	Curled	Concentric	White
D	6 mm	Round	Smooth	Smooth	Off-White
E	14 mm	Irregular	Lobate	Contoured	Mustard



Heterotrophic plate count (HPC)

Practical exercise

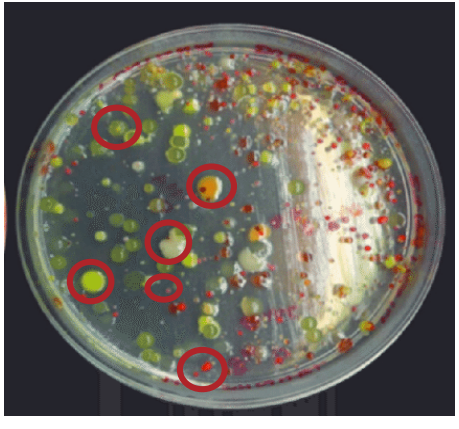
14:00 – 14:15	**Group 1**
14:15 – 14:30	**Group 2**
14:30 – 14:45	**Group 3**
14:45 – 15:00	**Group 4**

Meanwhile: **https://www.quia.com/rr/220195.html**

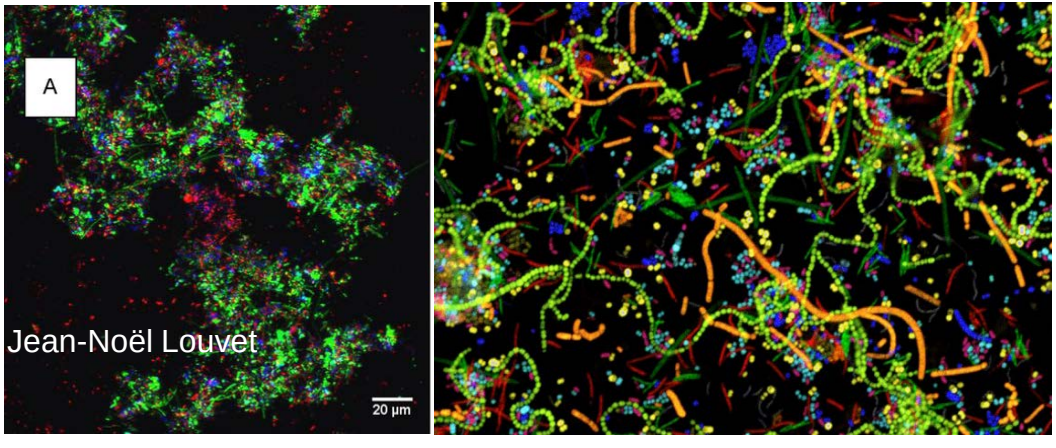
Unculturable bacteria

- current laboratory culturing techniques are unable to grow most of the bacteria in the laboratory
- culture-independent methods bring more information on microbial composition in environmental samples

Six different types of colonies



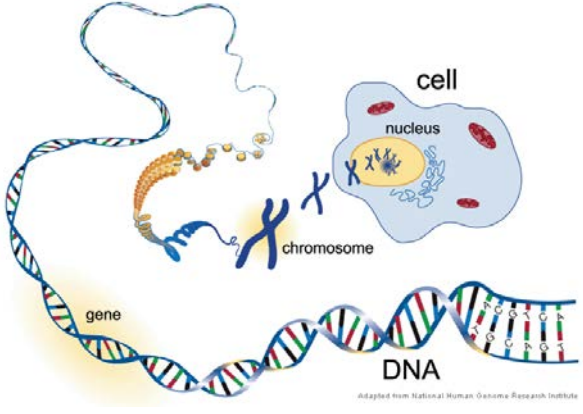
Activated sludge flocs

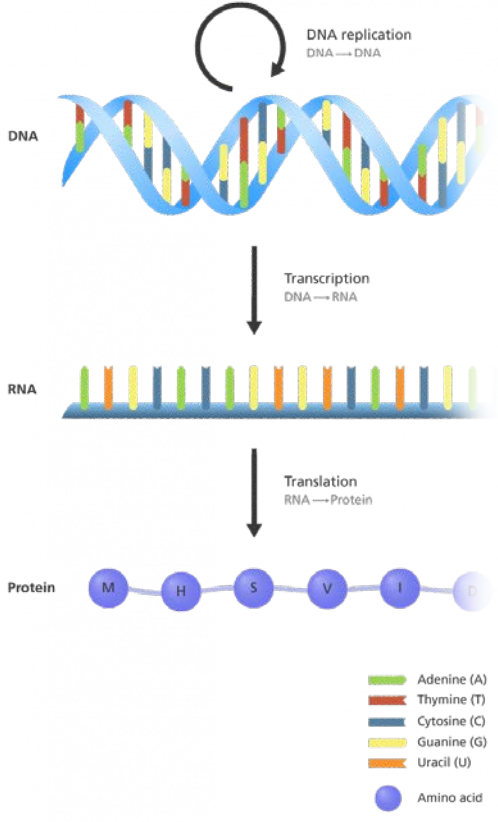


the content in activated sludge is 10^{10}- 10^{12} cells/L

Culture-independent methods

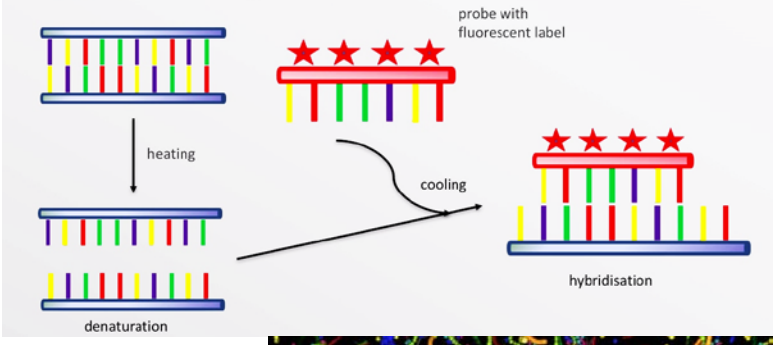
- All known organisms use DNA as genetic material
- The genetic code is universal
- Gene sequences in different organisms express the same proteins





Culture-independent methods

1. Fluorescent In Situ Hybridization (FISH)

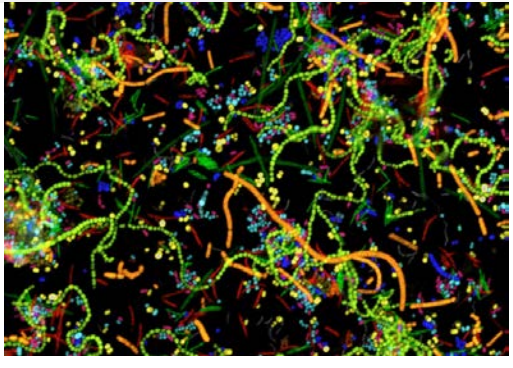


2. 16S rRNA

Activated Sludge:
- Protozoa
- Bacteria
- viruses, archaea etc.

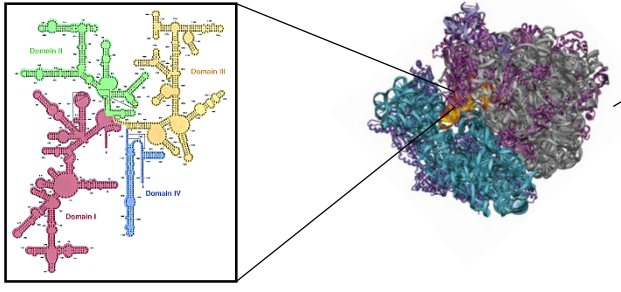
Total DNA

Prokaryotic DNA

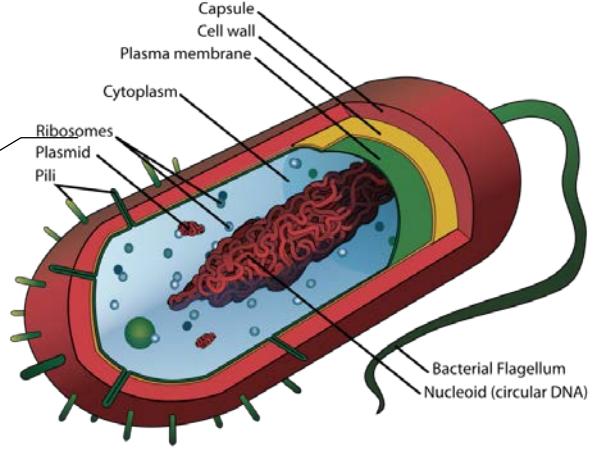


16S ribosomal RNA (16S rRNA)

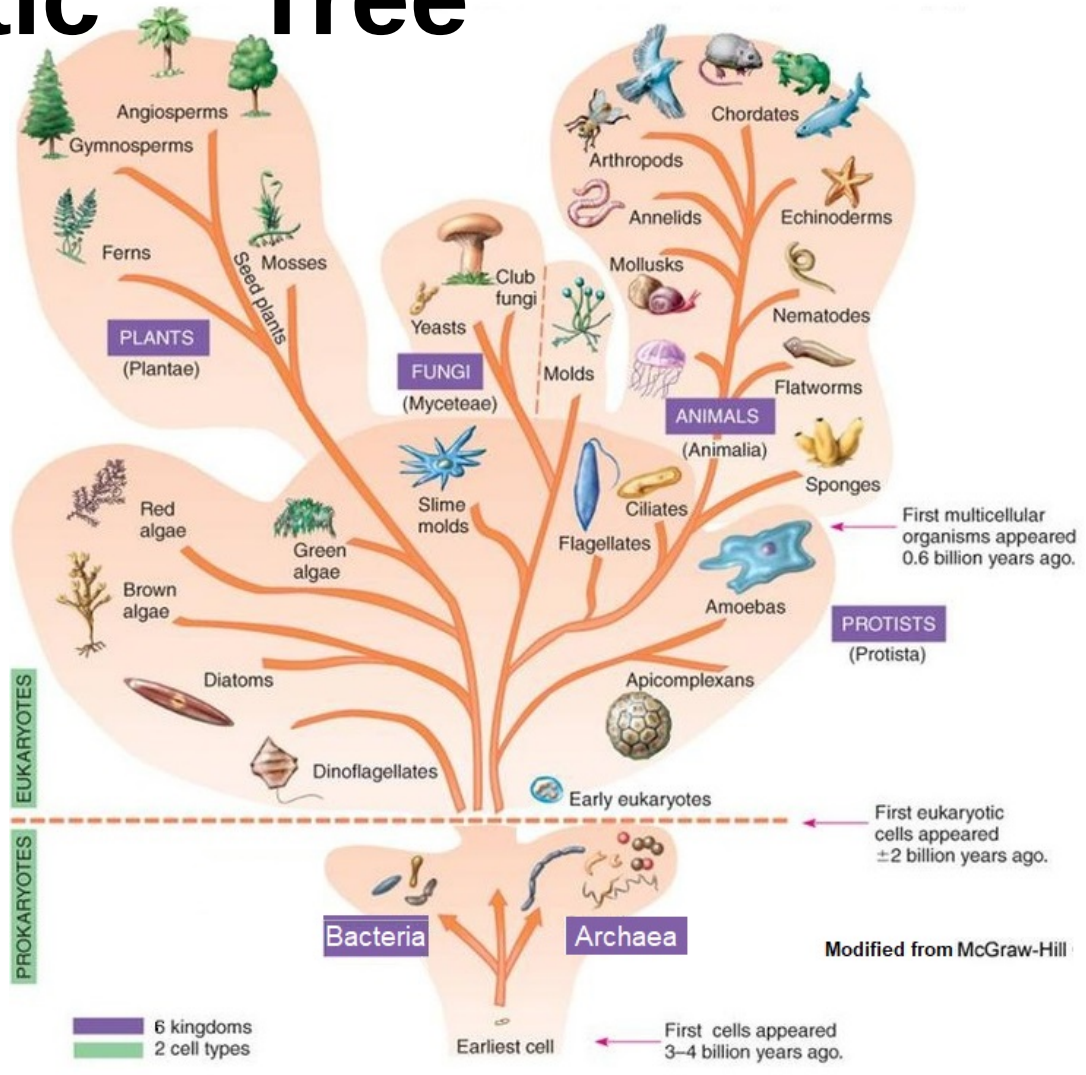
- has proved to be the most informative for investigating evolutionary relatedness
- used extensively in the classification and identification of *Bacteria* and *Archaea*
- ribosome – molecular structure (15000 in each *E. coli* cell , 10 mln in eucariotic cell)



16S rRNA



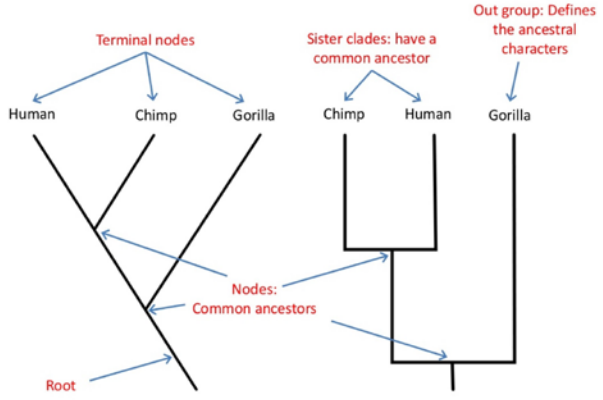
Phylogenetic Tree

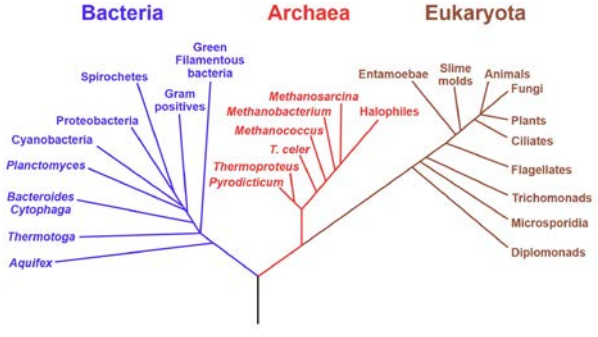


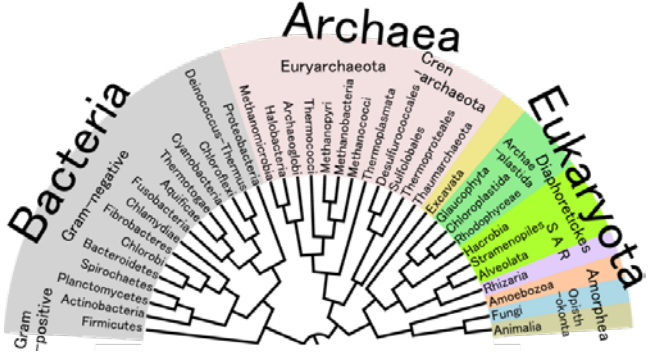
Phylogenetic tree

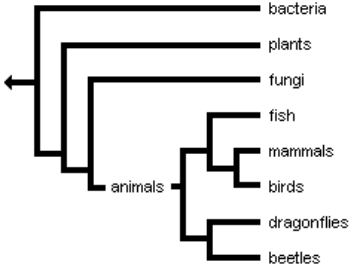
- DNA and the amino acids they code for are the primary basis for grouping organisms into clades (clade is a group of organisms evolved from the common ancestor)

- Evidence of which species are part of which clade can be obtained from the base of sequences of genes or the corresponding amino acid sequence of a protein





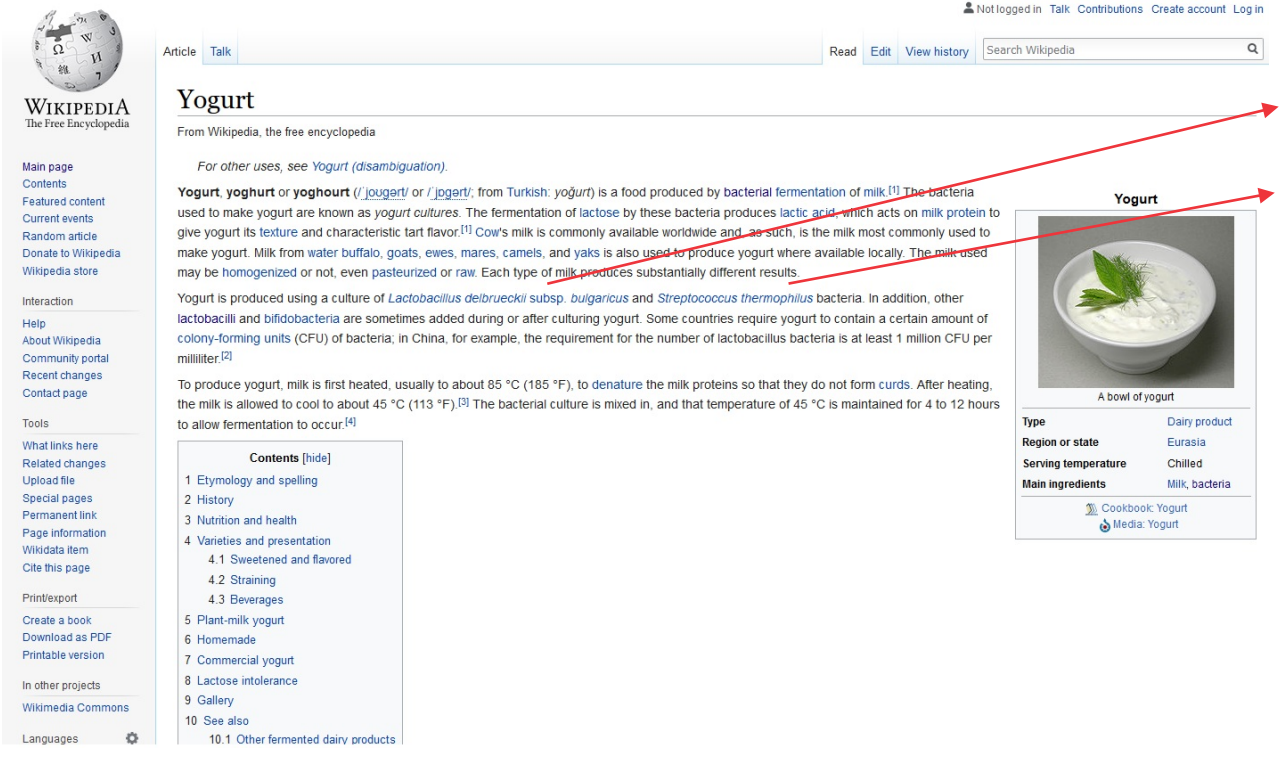




Sequence differences accumulate gradually so there is a positive correlation between the number of differences between two species and the time since they diverged from common ancestor

Example

Phylogenetic tree of yogurt bacteria



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Yogurt

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For other uses, see Yogurt (disambiguation).

Yogurt, **yoghurt** or **yoghourt** (/ˈjoʊgərt/ or /ˈjɒgərt/; from Turkish: *yoğurt*) is a food produced by bacterial fermentation of milk.[1] The bacteria used to make yogurt are known as *yogurt cultures*. The fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give yogurt its texture and characteristic tart flavor.[1] Cow's milk is commonly available worldwide and, as such, is the milk most commonly used to make yogurt. Milk from water buffalo, goats, ewes, mares, camels, and yaks is also used to produce yogurt where available locally. The milk used may be homogenized or not, even pasteurized or raw. Each type of milk produces substantially different results.

Yogurt is produced using a culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* bacteria. In addition, other lactobacilli and bifidobacteria are sometimes added during or after culturing yogurt. Some countries require yogurt to contain a certain amount of colony-forming units (CFU) of bacteria; in China, for example, the requirement for the number of lactobacillus bacteria is at least 1 million CFU per milliliter.[2]

To produce yogurt, milk is first heated, usually to about 85 °C (185 °F), to denature the milk proteins so that they do not form curds. After heating, the milk is allowed to cool to about 45 °C (113 °F).[3] The bacterial culture is mixed in, and that temperature of 45 °C is maintained for 4 to 12 hours to allow fermentation to occur.[4]

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2 History
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4.1 Sweetened and flavored
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7 Commercial yogurt
8 Lactose intolerance
9 Gallery
10 See also
10.1 Other fermented dairy products

Yogurt

A bowl of yogurt

Type	Dairy product
Region or state	Eurasia
Serving temperature	Chilled
Main ingredients	Milk, bacteria

Cookbook: Yogurt
Media: Yogurt

Lactobacillus bulgaricus

Streptococcus thermophilus

Streptococcus salivarius

Lactobacillus acidophilus

Lactobacillus casei

Bifidobacterium adolescentis

Bifidobacterium bifidum

Yogurt bacteria 16S rRNA partial sequences

>Lactobacillus-bulgaricus
1 gcagtcgagc gagctgaatt caaagatccc ttcggggtga tttgttggac gctagcggcg
61 gatgggtgag taacacgtgg gcaatctgcc ctaaagactg ggataccact tggaaacagg
121 tgctaatacc ggataacaac atgaatcgca tgattcaagt ttgaaaggcg gcgtaagctg
181 tcactttagg atgagcccgc ggcgcattag ctagttggtg gggtaaaggc ctaccaaggc
241 aatgatgcgt agccgagttg agagactgat cggccacatt gggactgaga cacggcccaa
301 actcctacgg gaggcagcag tagggaatct tccacaatgg acgcaagtct gatggagcaa
361 cgccgcgtga gtgaagaagg ttttcggatc gtaaagctct gttgttggtg aagaaggata
421 gaggcagtaa ctggtcttta tttgacggta atcaaccaga aagtcacggc taactacgtg
481 ccagcagccg cggtaatacg taggtggcaa gcgttgtccg gatttattgg gcgtaaagcg
541 agcgcaggcg gaatgataag tctgatgtga aagcccacgg ctcaaccgtg gaactgcatc
601 ggaaactgtc attcttgagt gcagaagagg agagtggaat tccatgtgta gcggtggaat

>Streptococcus-thermophilus
1 tgcaagtaga acgctgaaga gaggagcttg ctcttcttgg atgagttgcg aacgggtgag
61 taacgcgtag gtaacctgcc ttgtagcggg ggataactat tggaaacgat agctaatacc
121 gcataacaat ggatgacaca tgtcatttat ttgaaagggg caattgctcc actacaagat
181 ggacctgcgt tgtattagct agtaggtgag gtaatggctc acctaggcga cgatacatag
241 ccgacctgag agggtgatcg gccacactgg gactgagaca cggcccagac tcctacggga
301 ggcagcagta gggaatcttc ggcaatgggg gcaaccctga ccgagcaacg ccgcgtgagt
361 gaagaaggtt ttcggatcgt aaagctctgt tgtaagtcaa gaacgggtgt gagagtggaa
421 agttcacact gtgacggtag cttaccagaa agggacggct aactacgtgc cagcagccgc
481 ggtaatacgt aggtcccgag cgttgtccgg atttattggg cgtaaagcga gcgcaggcgg
541 tttgataagt ctgaagttaa aggctgtggc tcaaccatag ttcgctttgg aaactgtcaa
601 acttgagtgc agaaggggag agtggaattc catgtgtagc ggtgaaatgc gtagatatat
661 ggaggaacac cggtggcgaa agcggctctc tggtctgtaa ctgacgctga ggctcgaaag

>Lactobacillus-casei
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61 tgcttgcacc gagattcaac atggaacgwg tgncggacgg gtgagtaaca cgtgggtaac
121 ctgcccttaa gtgggggata acatttggaa acagatgcta ataccgcata gatccaagaa
181 ccgcatggtt cttggctgaa agatggcgta agctatcgct tttggatgga cccgcggcgt
241 attagctagt tggtgaggta atggctcacc aaggcgatga tacgtagccg aactgagagg
301 ttgatcggcc acattgggac tgagacacgg cccaaactct acgggaggca gcagtaggga
361 atcttccaca atggacgcaa gtctgatgga gcaacgccgc gtgagtgaag aaggctttcg
421 ggtcgtaaaa ctctgttgtt ggagaagaat ggtcggcaga gtaactgttg tcggcgtgac
481 ggtatccaac cagaaagcca cggctaacta cgtgccagca gccgcggtaa tacgtaggtg
541 gcaagcgtta tccggattta ttgggcgtaa agcgagcgca ggcggttttt taagtctgat
601 gtgaaagccc tcggcttaac cgaggaagcg catcggaaac tgggaaactt gagtgcagaa

>Bifidobacterium-adolescentis
1 agagttgatc cggctcagga tgaacgcggc ggcgtgctta acacatgcaa gtcgaacggg
61 atcccaggag cttgctcctg ggtgagagtg gcgaacgggt gagtaatgcg tgaccgacct
121 gccccataca ccggaatagc tcctggaaac gggtggtaat gccggatgct ccacctgacc
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541 gtaaagggct cgtaggcggt tcgtcgcgtc cggtgtgaaa gtccatcgct taacggtgga
601 tccgcgccgg gtacgggcgg gcttgagtgc ggtaggggag actggaattc ccggtgtaac

>Bifidobacterium-bifidum
1 aacgggatcc atcaagcttg cttggtggtg agagtggcga acgggtgagt aatgcgtgac
61 cgacctgccc catgctccgg aatagctcct ggaaacgggt ggtaatgccg gatgttccac
121 atgatcgcat gtgattgtgg gaaagatttc atcggcgtgg gatggggtcg cgtcctatca
181 gcttgttggt gaggtaacgg ctcaccaagg cttcgacggg tagccggcct gagagggcga
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301 ttgcacaatg ggcgcaagcc tgatgcagcg acgccgcgtg agggatggag gccttcgggt
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541 gtggatctgc gccgggtacg ggcgggctgg agtgcggtag gggagactgg aattcccggt
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>Streptococcus-salivarius
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61 gctgaagaga ggagcttgct cttcttggat gagttgcgaa cgggtgagta acgcgtaggt
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361 gaatcttcgg caatgggggc aaccctgacc gagcaacgcc gcgtgagtga agaaggtttt
421 cggatcgtaa agctctgttg taagtcaaga acgagtgtga gagtggaaag ttcacactgt
481 gacggtagct taccagaaag ggacggctac tacgtgcca gcagccgcgg taatacgtag
541 gtcccgagcg ttgtccggat ttattgggcg taaagcgagc gcaggcggtt tgataagtct
601 gaagttaaag gctgtggctc aaccatagtt cgctttggaa actgtcaaac ttgagtgcag

>Lactobacillus-acidophilus
1 agagtttgat nntggctcag gacgaacgct ggcggcgtgc ctaatacatg caagtcgagc
61 gagcttgcct agatgatttt agtgcttgca ctaaatgaaa ctagatacaa gcgagcggcg
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301 atgatgcata gccgagttga gagactgatc ggccacattg ggactgagac acggcccaaa
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481 aggtagtaac tggcctttat ttgacggtaa ttacttagaa agtcacggct aactacgtgc
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601 gtgcaggcgg ttcaataagt ctgatgtgaa acgcttcggc tcaaccggag aattgcatca

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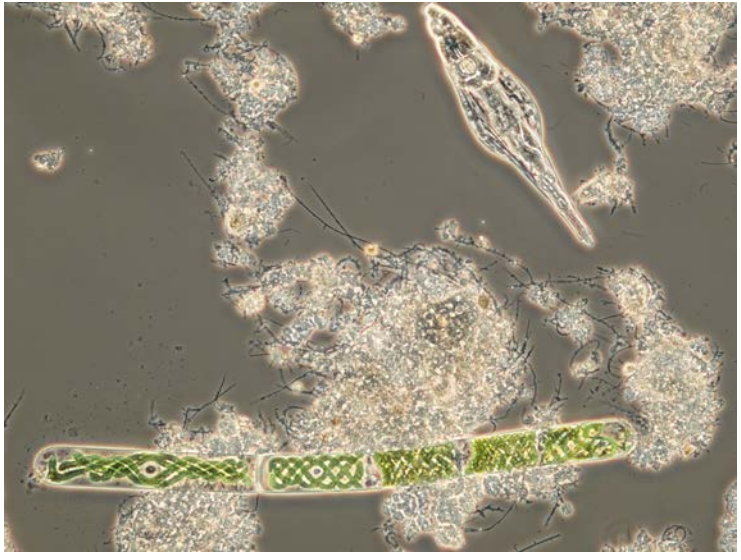
Microbial communities

Microbial communities are groups of microorganisms that share a common living space

- Resource competition
    - Extracellular enzimes
- Metabolic interactions:
    - Co-metabolism
    - Cross-feeding
    - Sequential utilization (nitrification)
    - Chemical modification of environment (pH)

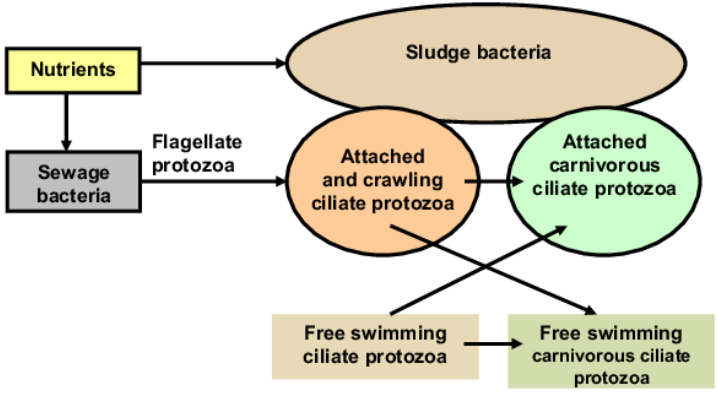
- Signaling
- Trophic level interactions
    - Parasitism

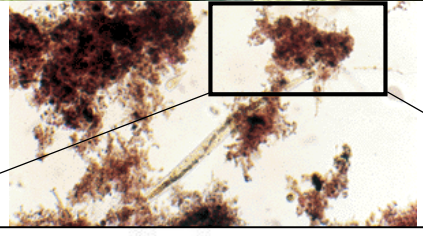
- Horizontal gene transfer
- Co-evolution



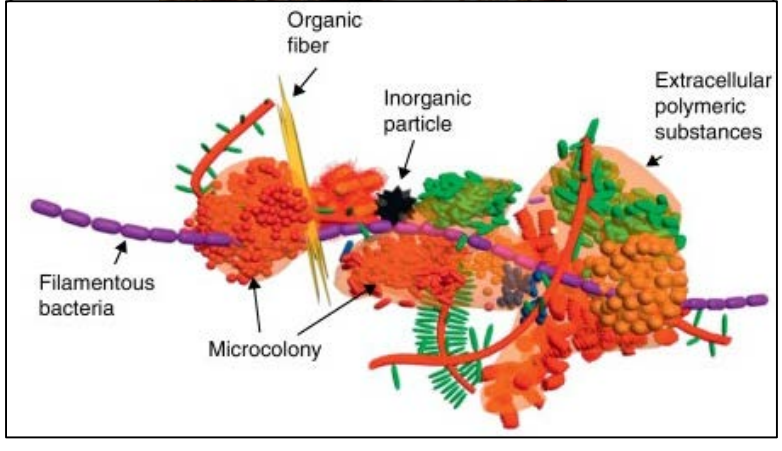
Activated Sludge

- Microorganisms are aggregated into *flocs*





0,1-3,0 mm

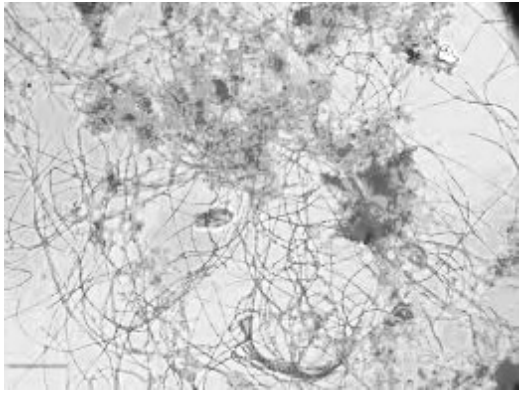


- granular activated sludge, MBR activated sludge, activated sludge biofilm, anaerobic activated sludge etc. have different microbial communities

Bulking and foaming

- can be caused by (excessive) formation of filamentous microorganisms

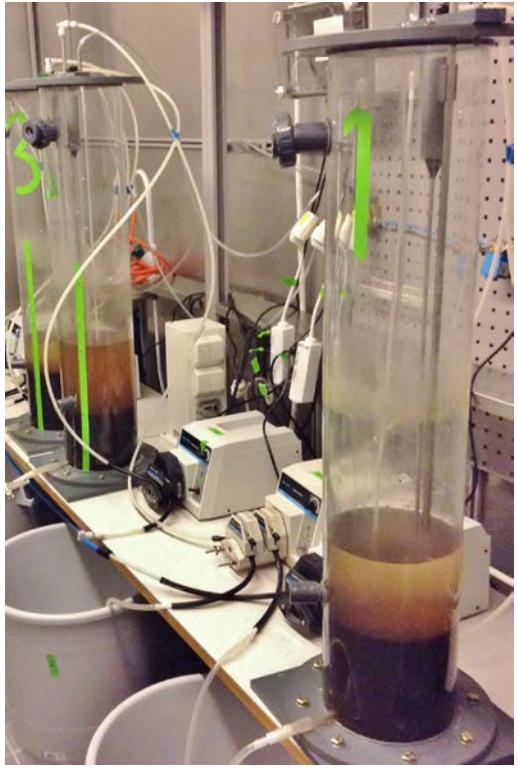
- normal flocs - a balance between floc-forming and filamentous microorganisms results in strong flocs that keep their integrity in the aeration basin and settle well in the sedimentation tank

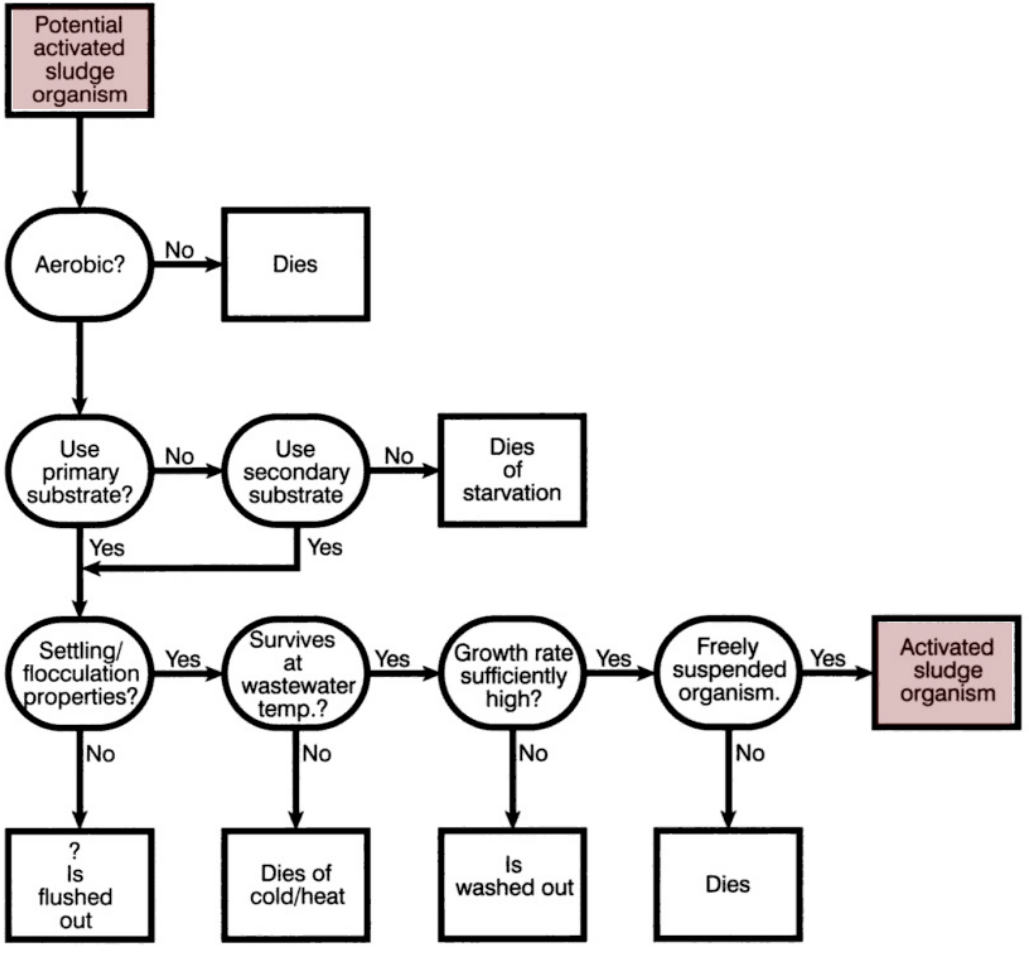


Activated sludge microbial community

Floc-forming	Foaming	Bulking
Zoogloea ramigera	*Nocardia (Gordona) amarae*	*Sphaerotilus natans*
Pseudomonas	*Microthrix parvicella*	*Microthrix parvicella*
Flavobacterium	*Rhodococcus spp.*	*Hatiscomenobacter hydrossis*
Micrococcus	*Skermania piniformis (Nocardia*	*Thiothrix spp., Beggiatoa*
Alcaligenes	*pinensis)*	*Nocardia spp.*
Bacillus	*Nocardia rhodochrous*	*Hydrogenophaga spp.*
Achromobacter	*Nocardia asteroides*	*Acidovorax spp.*
Corynebacterium	*Nocardia caviae*	*Nostocoida limicola*
Azotobacter	*Nocardia farcinica*	*Type 021N*
Nitrosomonas	*Tsukamurella paurometabolum*	*Type 1701*
Nitrobacter	*Zoogloea ramigera*	*Type 0411*
Acinetobacter	*Streptomyces spp.*	*Type 1863*
Comamonas	*Acinetobacter*	*Type 0675*
Desulfotomaculum	*Nostocoida limicola*	*Type 0041*
Desulfovibrio	*Type 1851 Micromonospora*	*Type 0803*
Thiobacterium	*Type 0675*	*Type 0092*
Thiothrix, Beggiatoa	*Type 0041*	*Type 0581*
Thiobacillus denitrificans	*Type 0803*	*Type 914*
Sarcina	*Type 0092*	
Pseudobacterium	*Type 0581*	
	Type 914	

Selection process for microbial community





Other examples of microbial communities