

Introduction to microbiology

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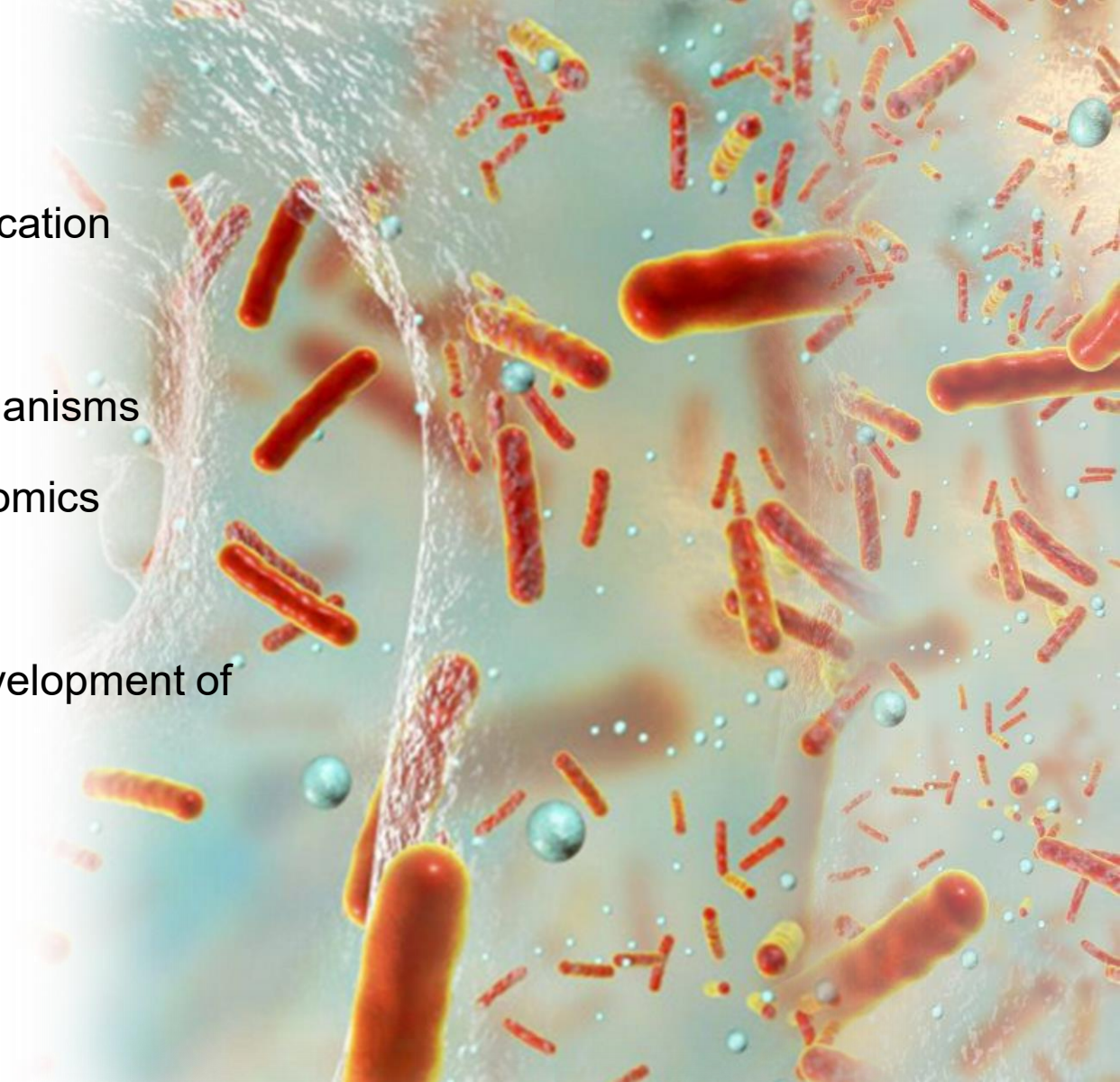
04.03.2021



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Aalto-universitetet
Aalto University

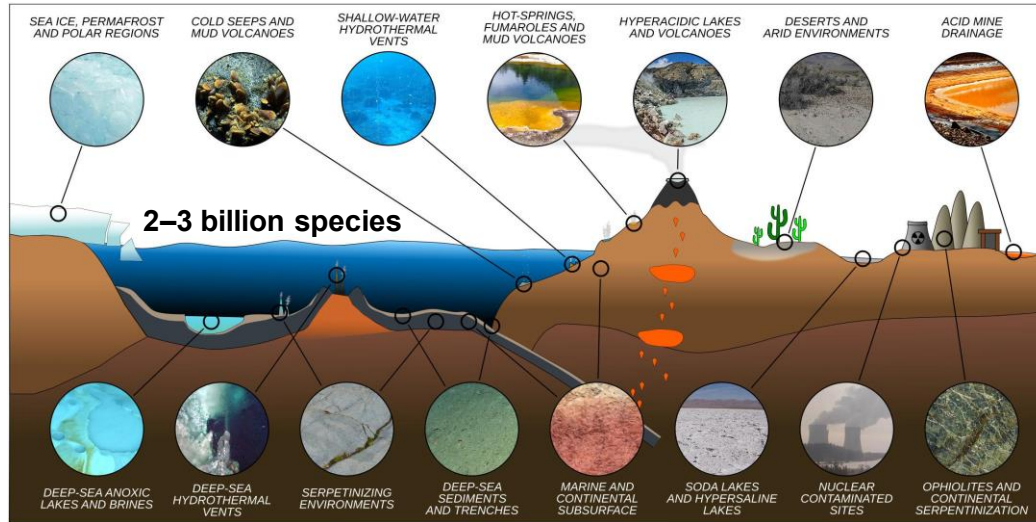
Outline

1. Terminology and classification
2. Microbial communities
3. Identification of microorganisms
4. Introduction to metagenomics
5. Practical exercise
6. Bacterial growth and development of bacterial community

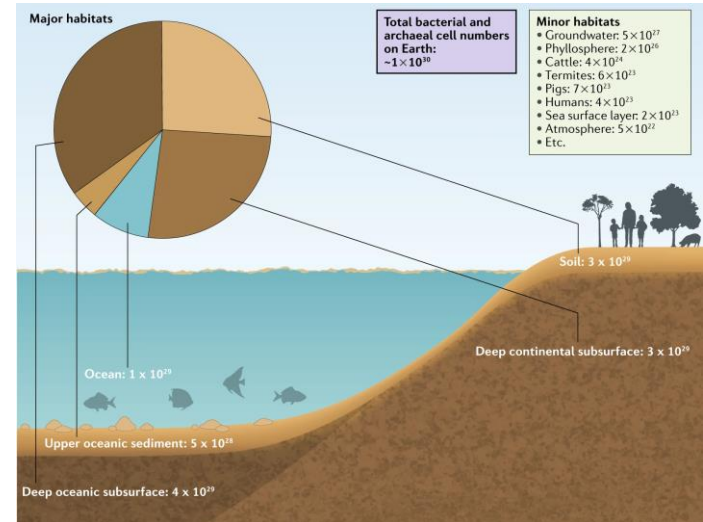


Microbiology

Microorganisms - all **living** organisms that are too small to be visible with the naked eye

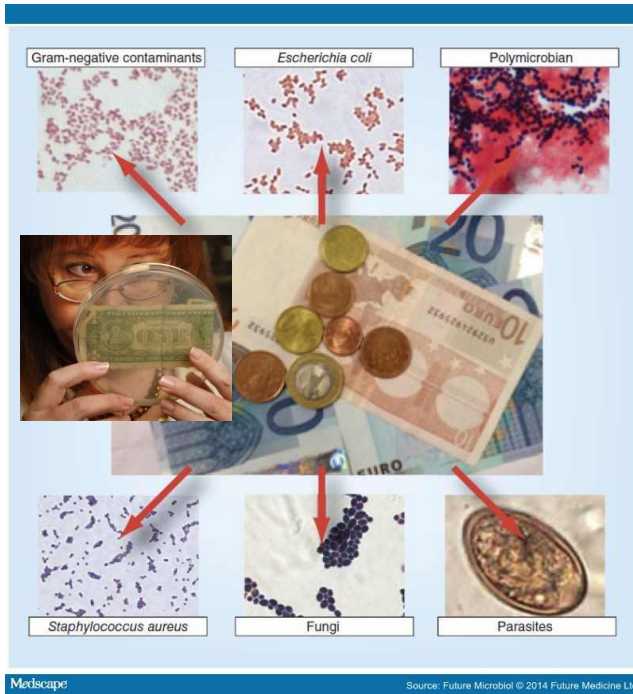


Merino et al., 2019



Hans-Curt Flemming & Stefan Wuerzt, 2019

According to estimation, 99% of the microorganisms on earth have not yet been identified!



Microbiology timeline

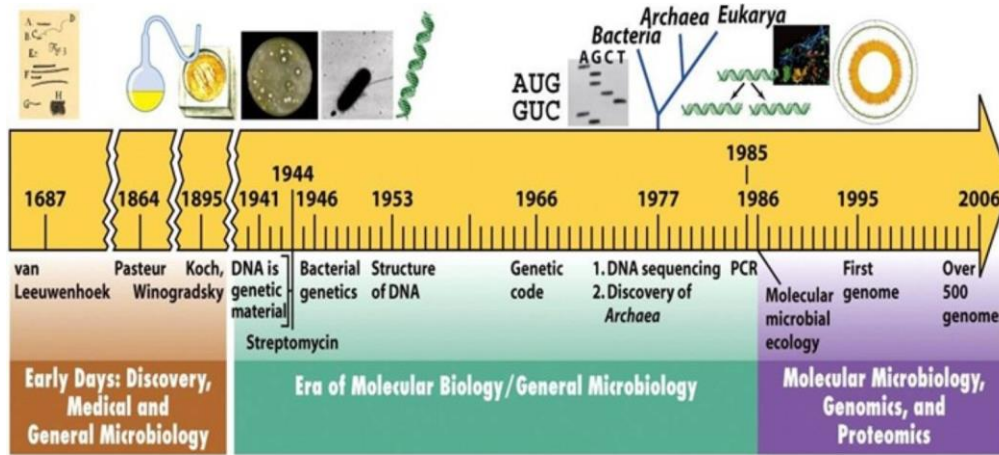
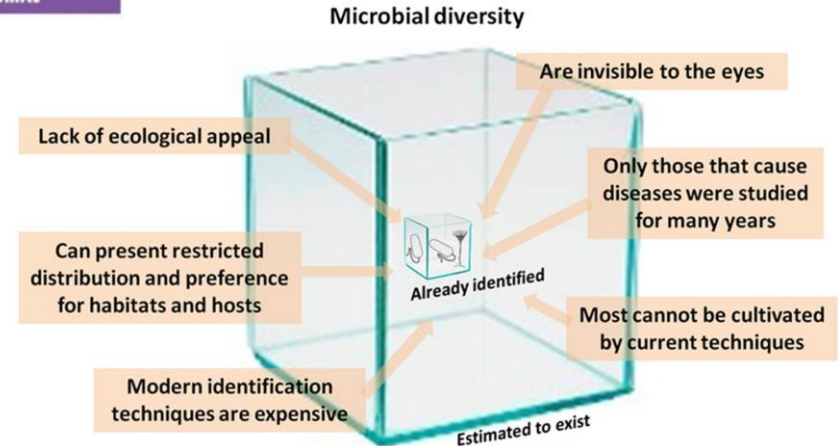
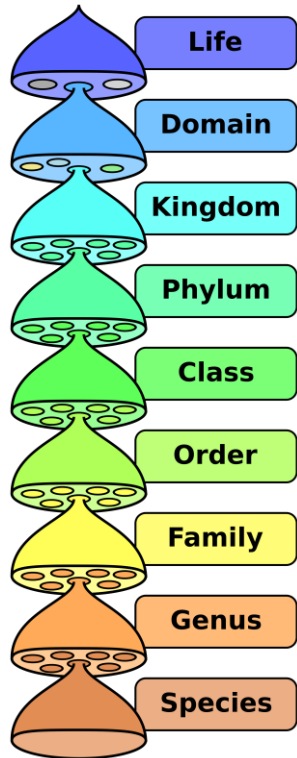


Figure 1-17 Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

Vitorino & Bessa, 2018



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, bacteria 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**

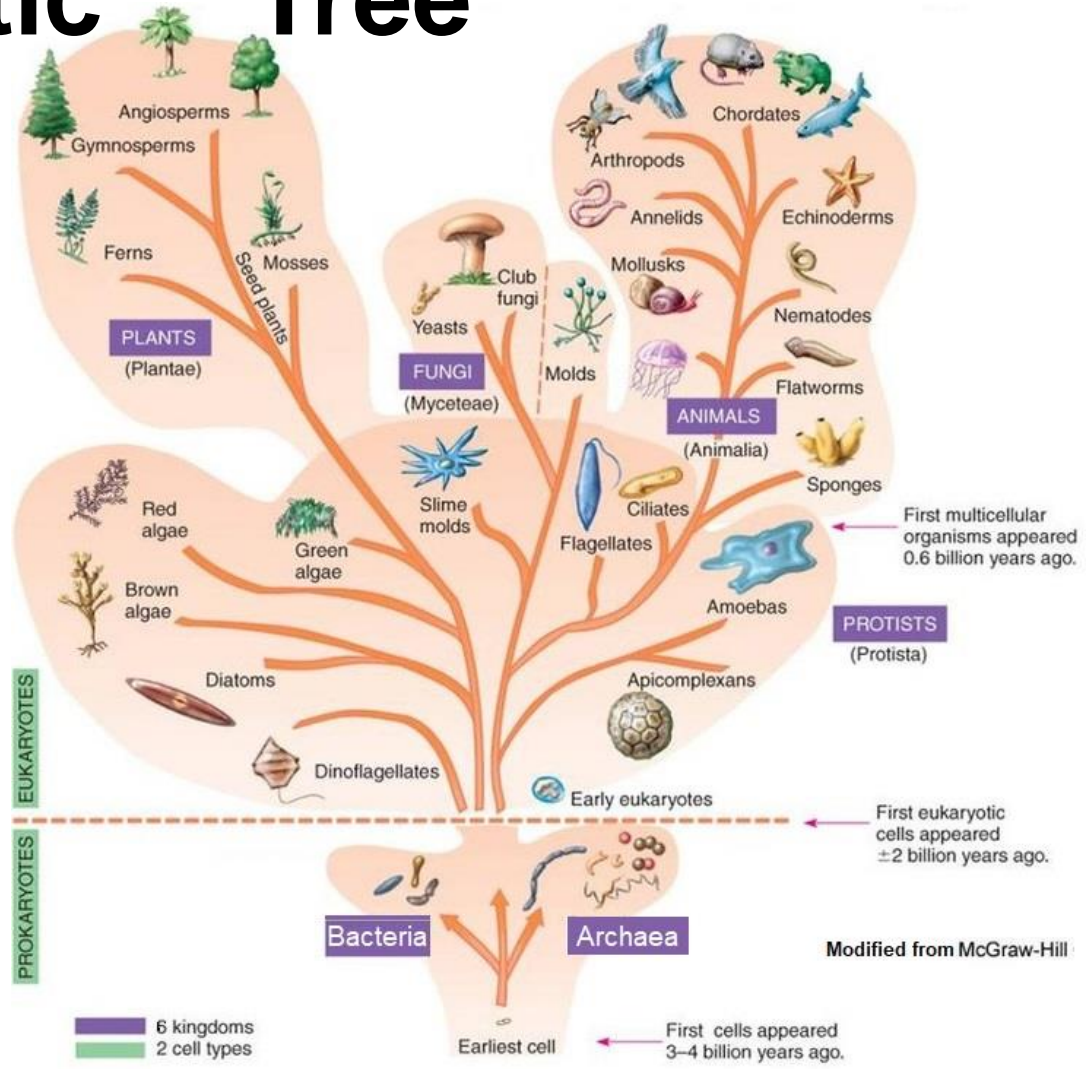
two name naming system

- 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

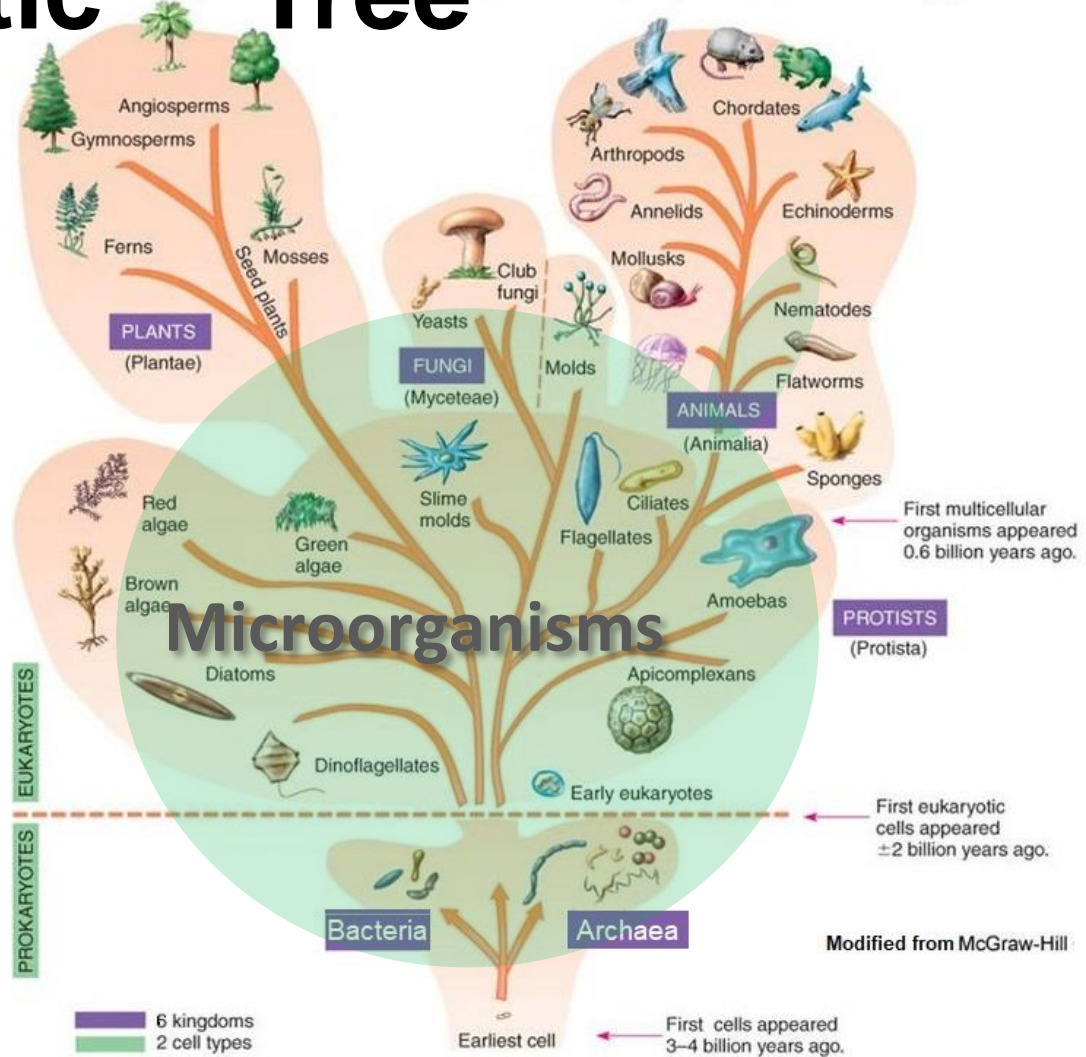
Classification of living organisms

Domain	Bacteria	Archaea	Eukarya			
Kingdom	Eubacteria	Archaeobacteria	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

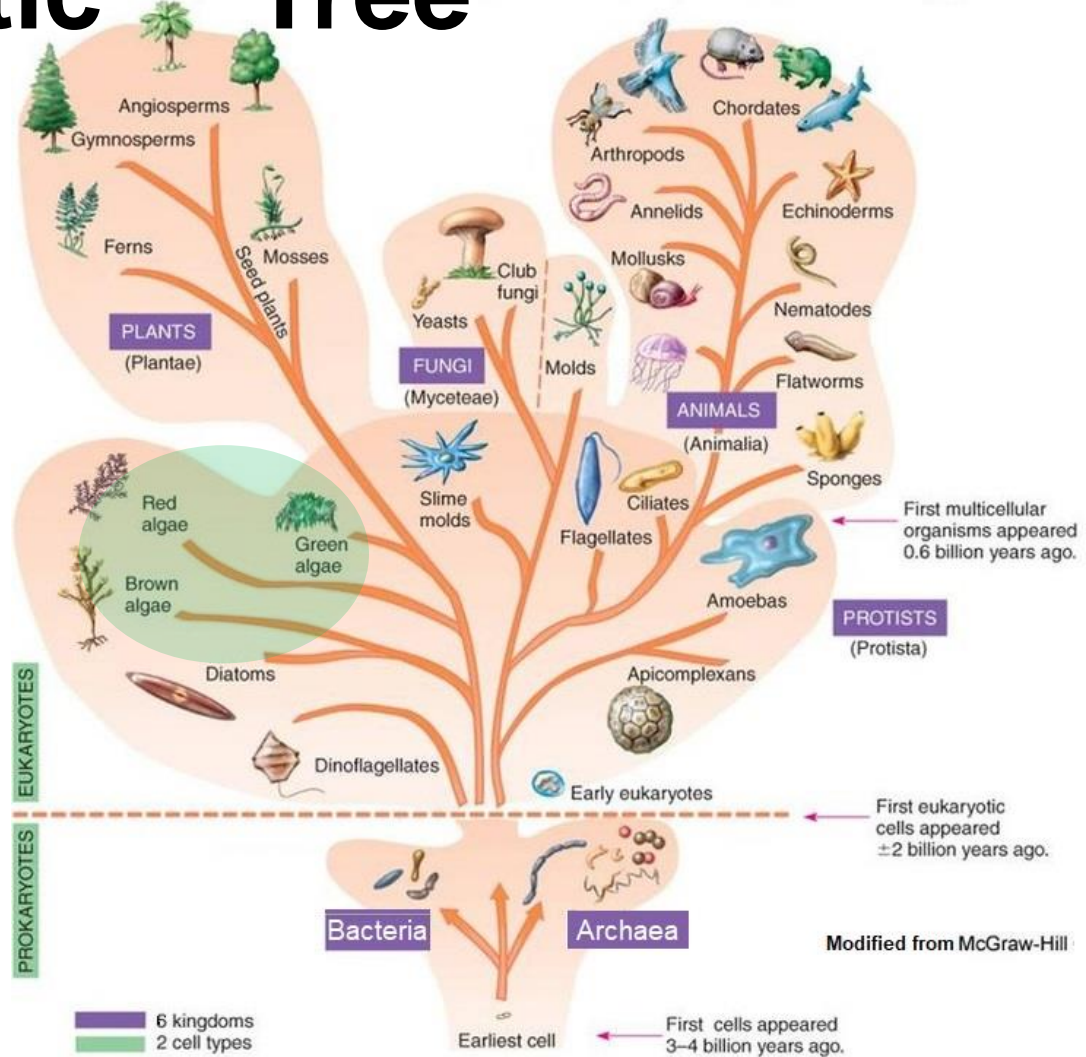
Phylogenetic Tree



Phylogenetic Tree

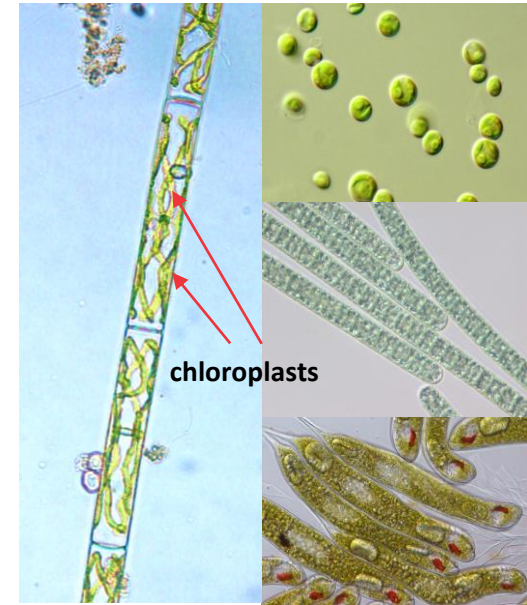


Phylogenetic Tree

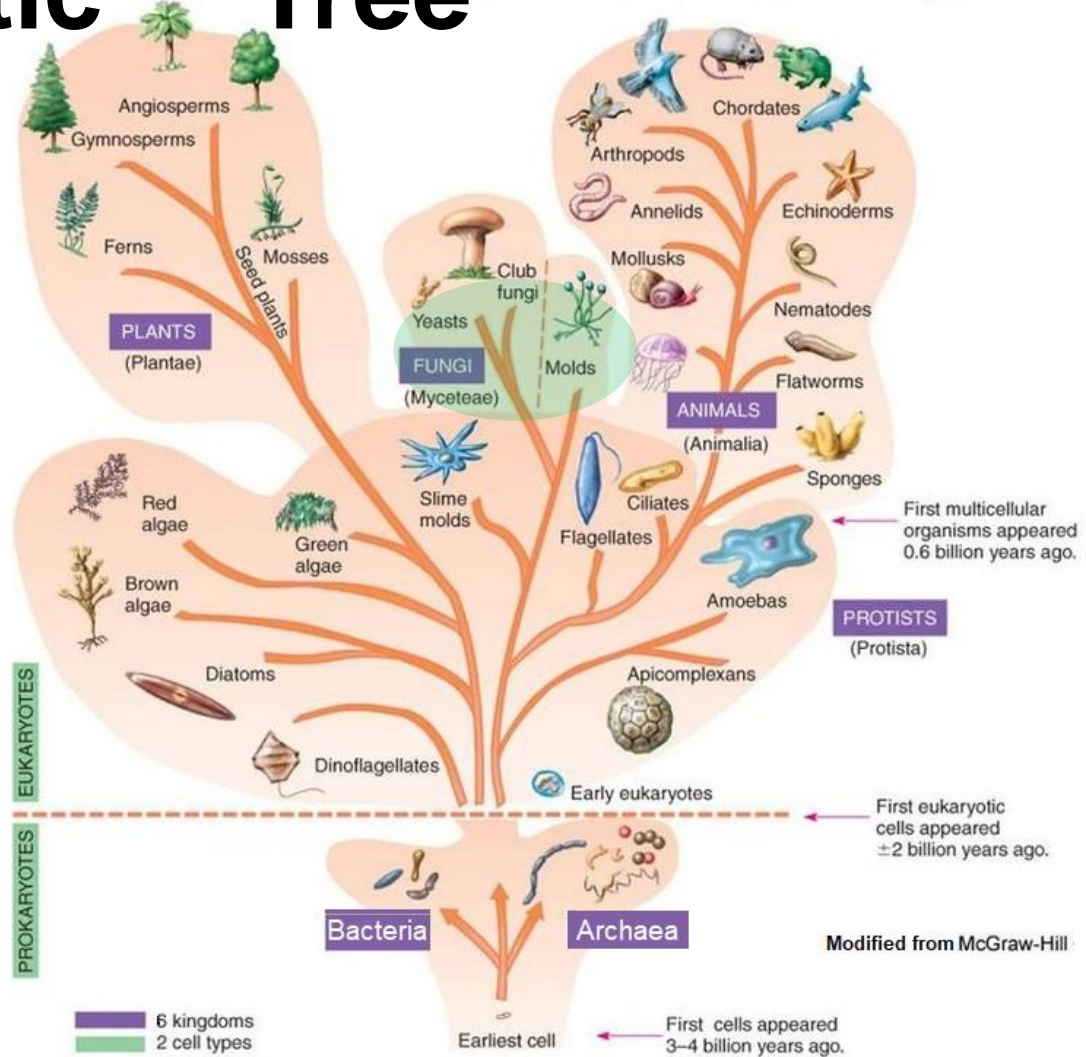


Algae

- 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

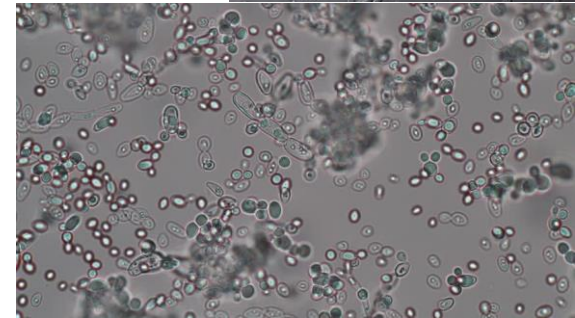
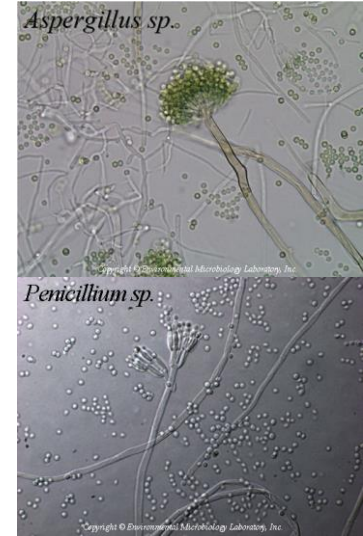


Phylogenetic Tree

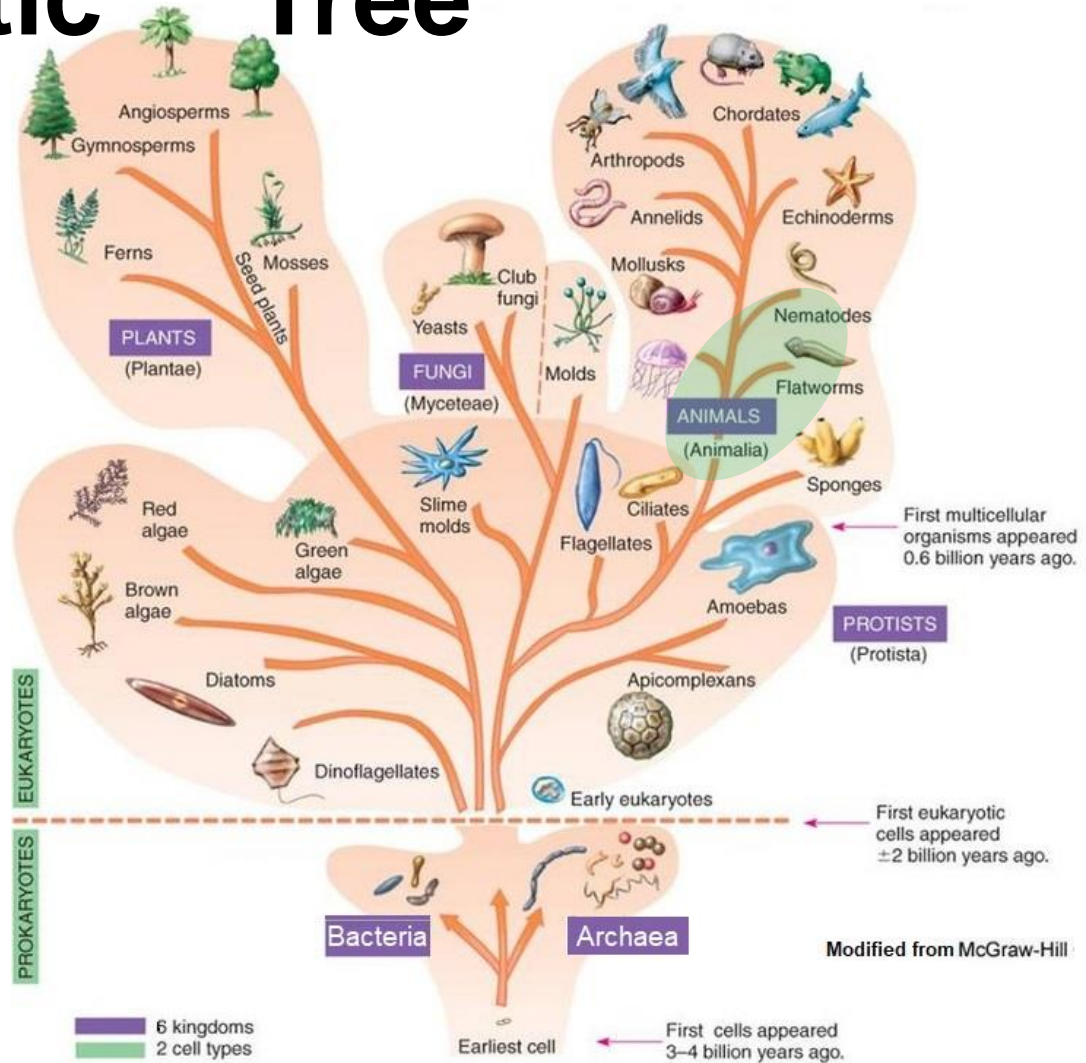


Fungi

- 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



Phylogenetic Tree



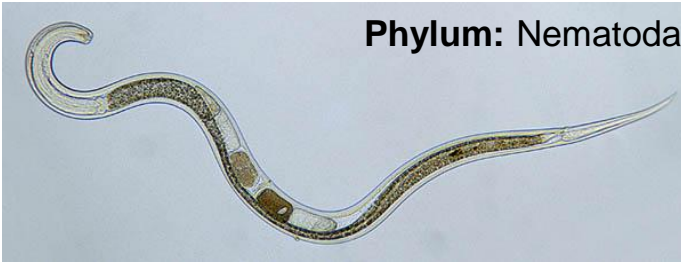
Metazoa

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

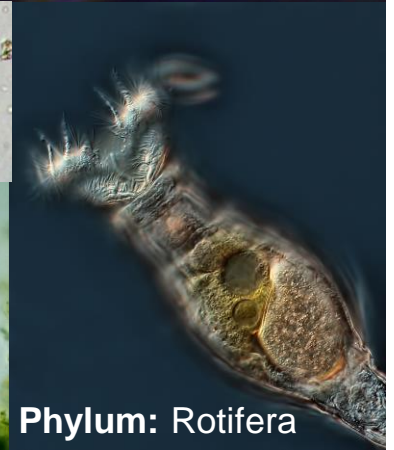
Phylum: Tardigrada



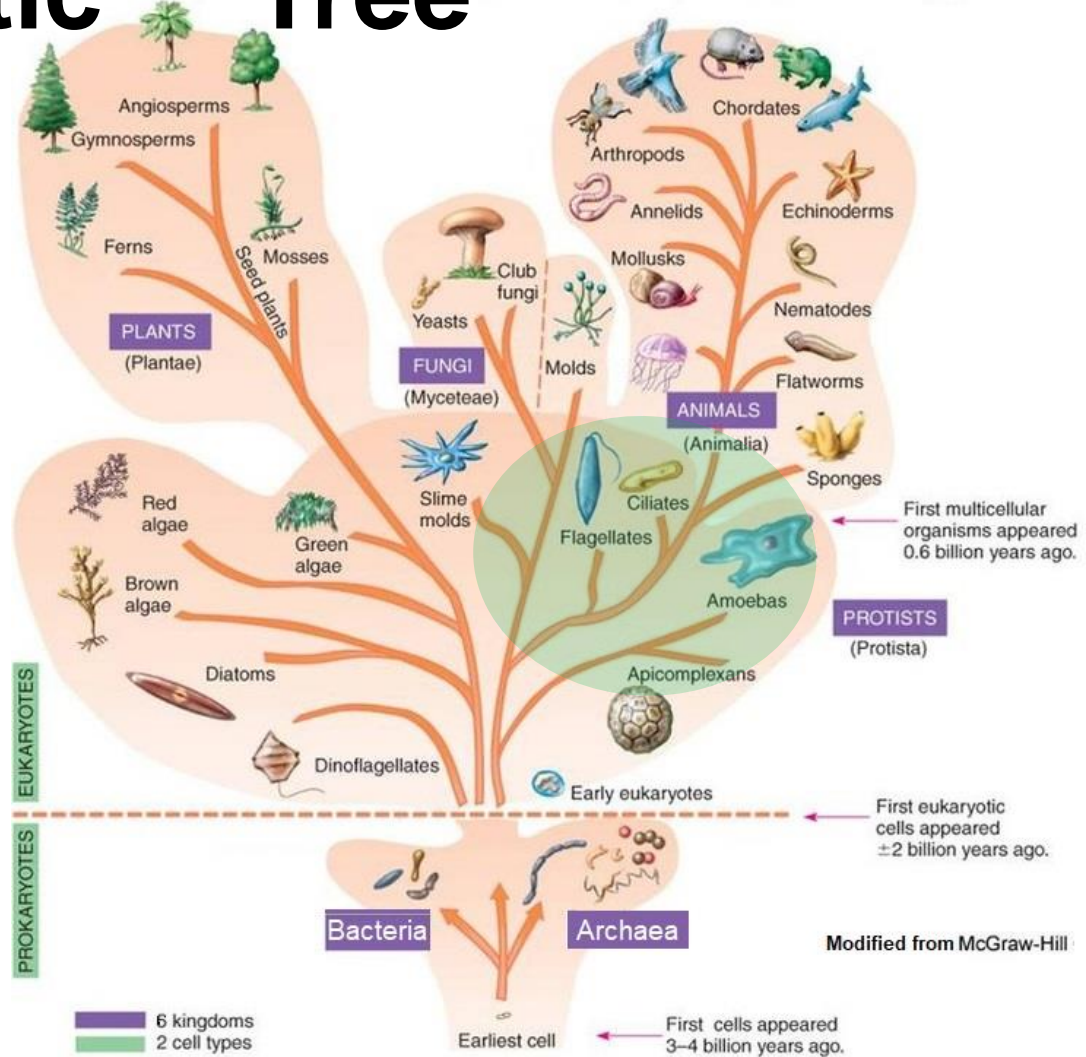
Phylum: Nematoda



Phylum: Rotifera



Phylogenetic Tree



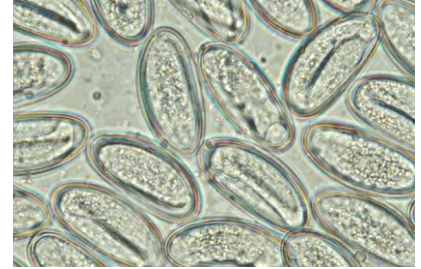
Modified from McGraw-Hill

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



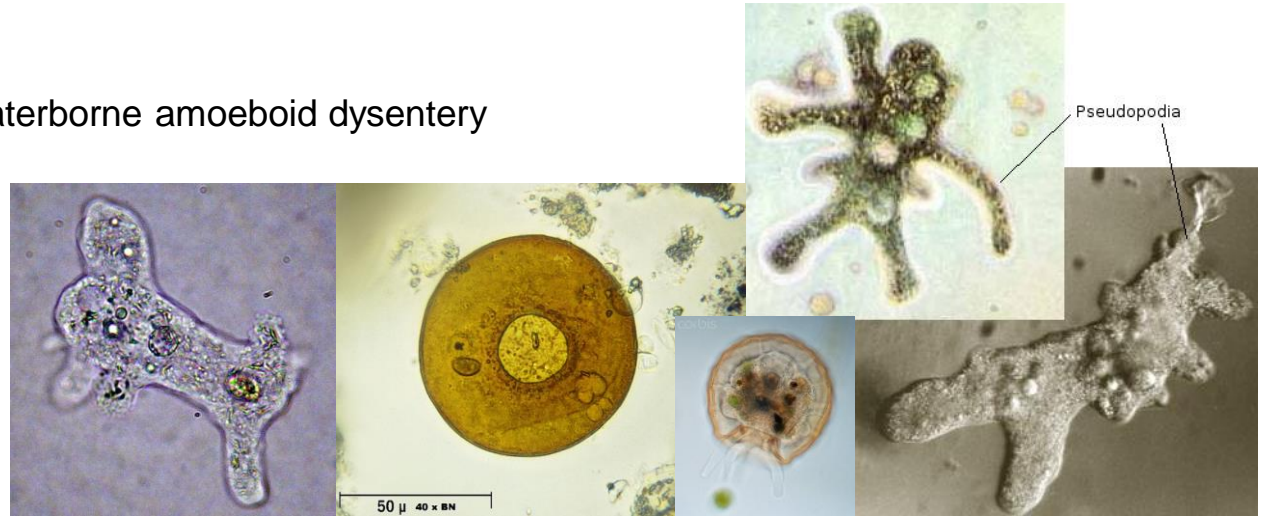
Giardia



Enterobius vermicularis eggs

Sarcodina

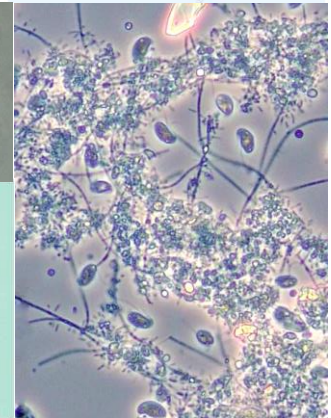
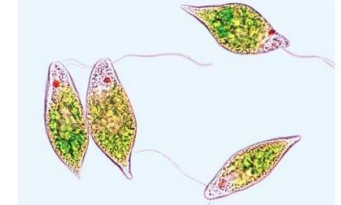
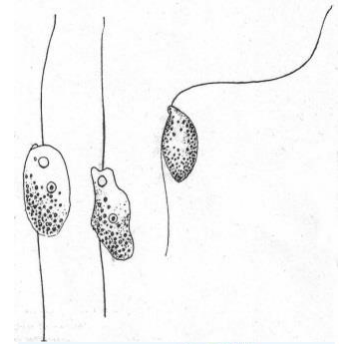
- the largest protozoan phylum: 11,500 living species and 33,000 fossil species
- move by cytoplasmic streaming in pseudopods (false “feet”)
- In activated sludge, indicate start-up conditions or recovery from toxicity, washout, and organic overload
- *Entamoeba histolytica* – waterborne amoeboid dysentery



Mastigophora (*Flagellates*)

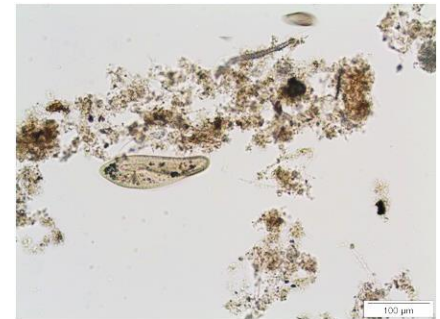
- one or more flagella (hair-like structure)
- free-living species are indicators of young activated sludge
- Several dangerous parasites

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



Ciliophora (*Ciliates*)

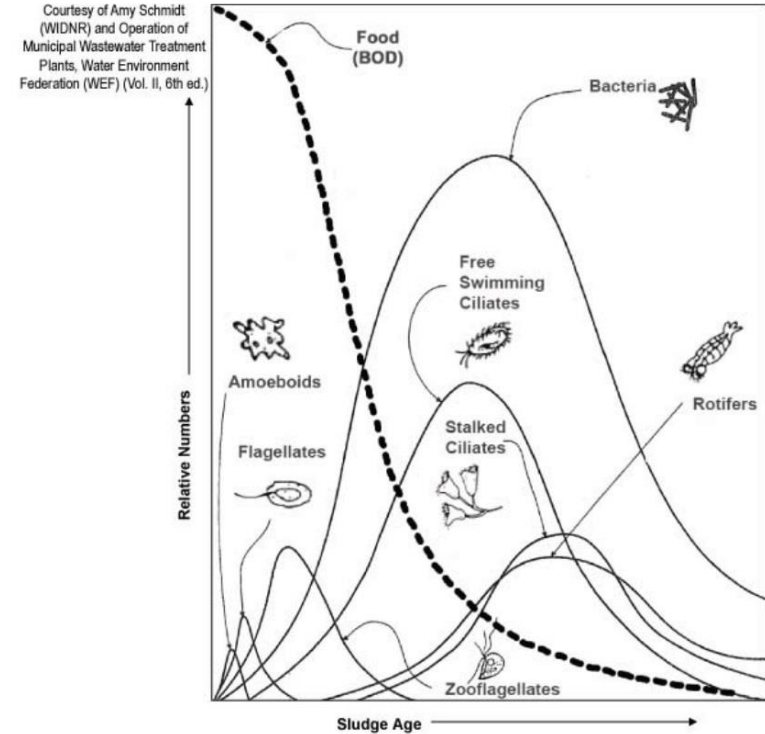
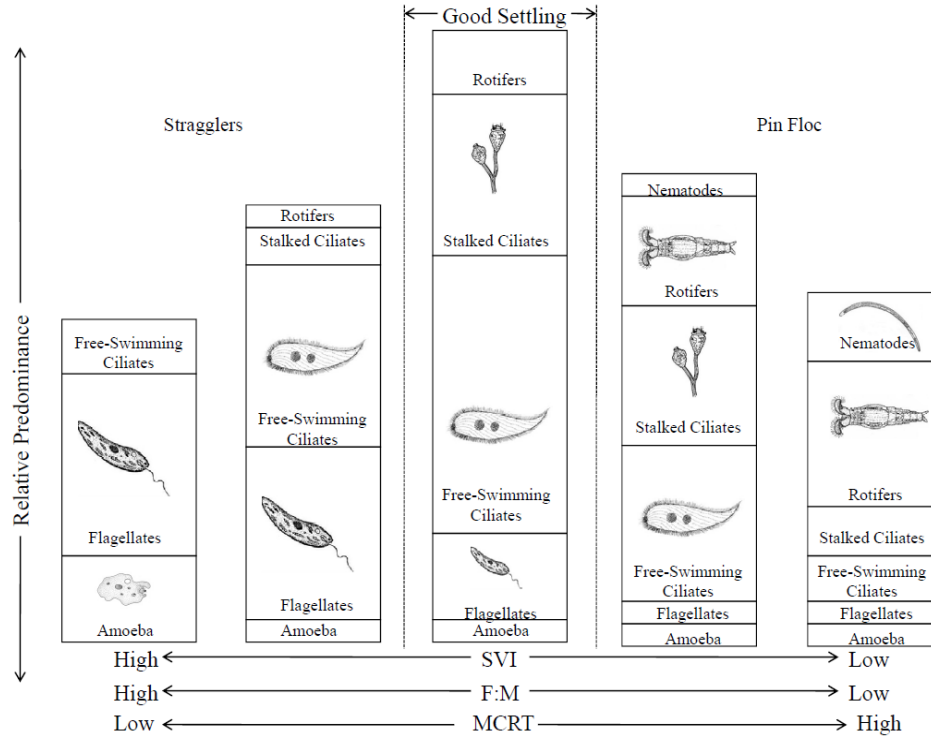
- phylum Ciliophora include ~8000 species
- move by cilia (multiple short hair-like structures)
- feeding mechanisms involve a mouth and cilia
- dominant in the presence of mature flocs and low BOD in the mixed liquor



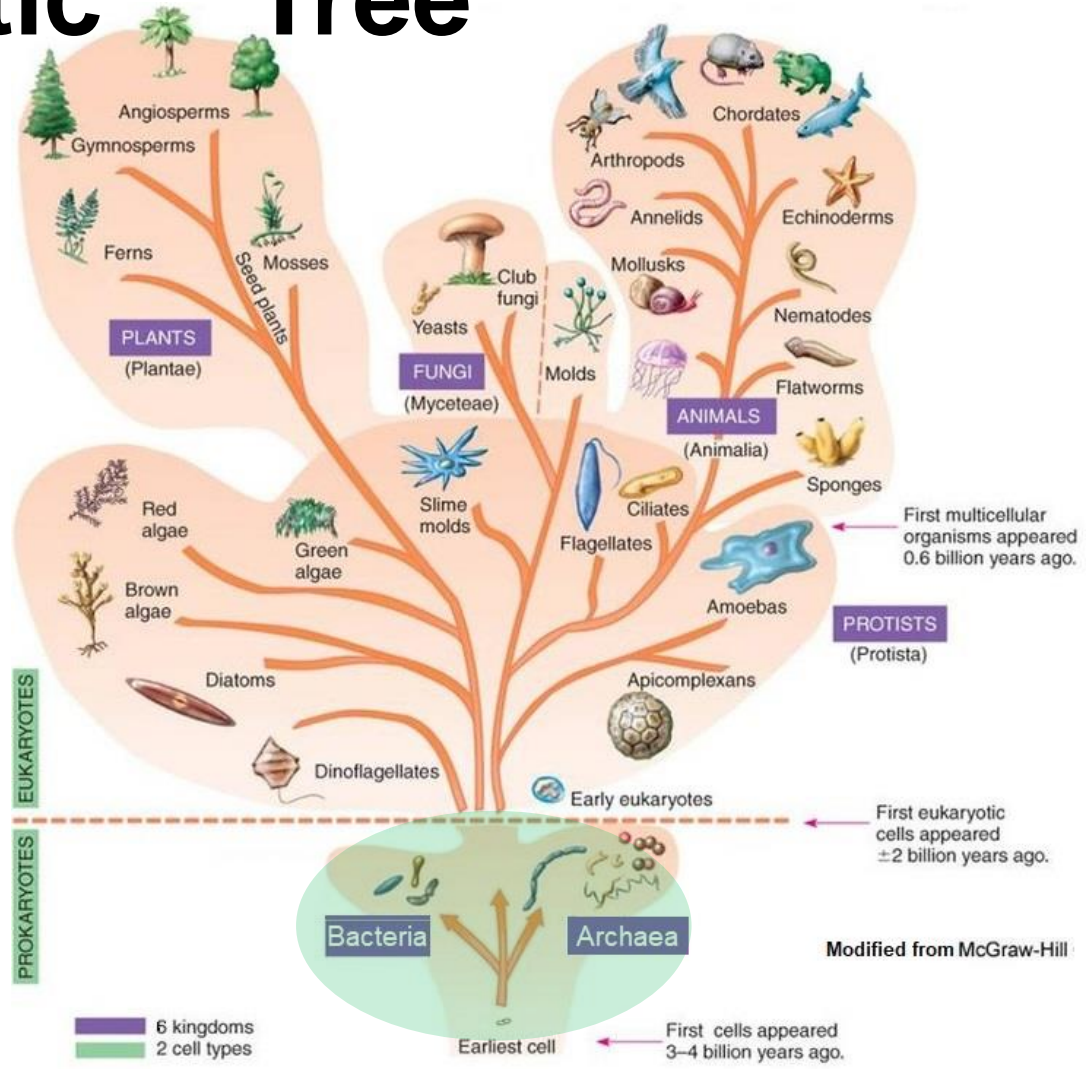
Size comparison



Wastewater treatment bioindicators

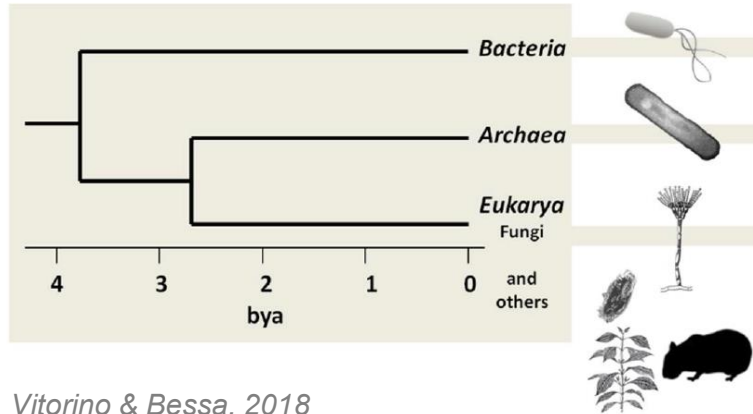


Phylogenetic Tree

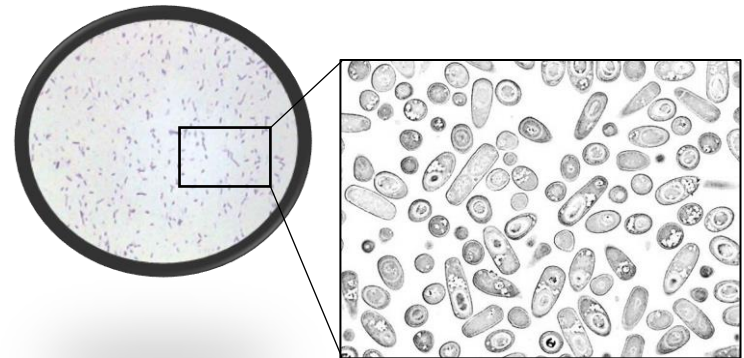


Bacteria and Archaea

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

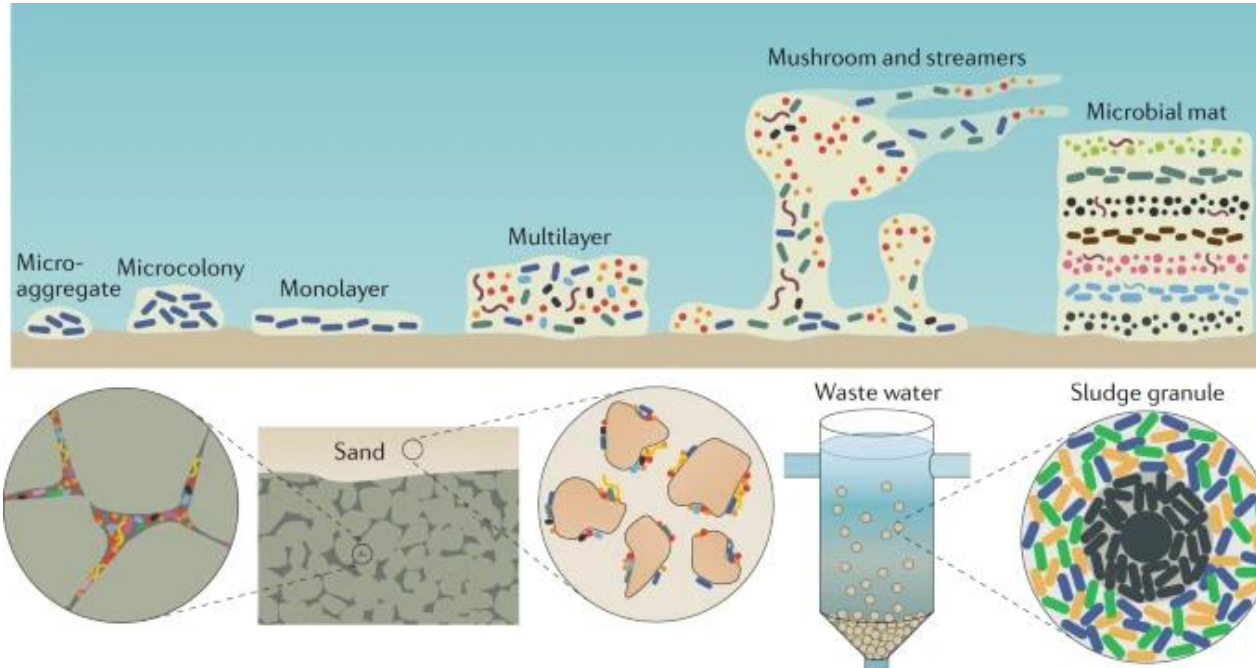


in oceans - 160 species/ml
in soils - 6400 - 38000 species/g
in sewage - 70 species/ml



Vitorino & Bessa, 2018

“Microbial cities”



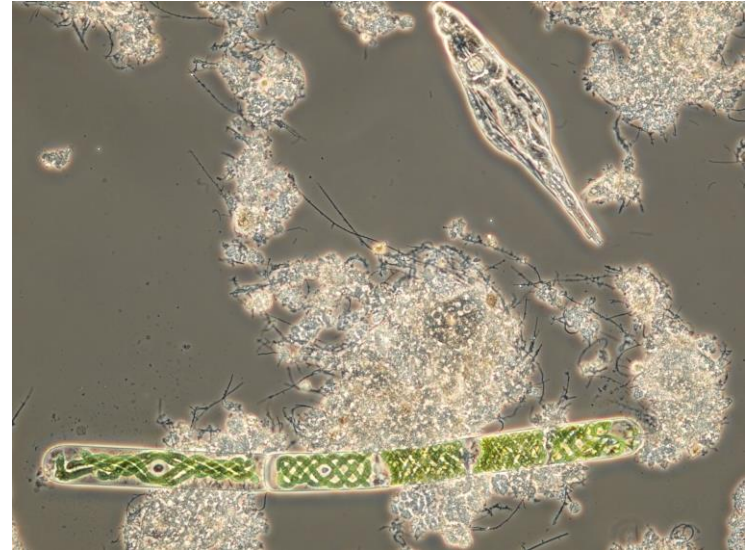
- Free living
- colonies
- flocs
- mats
- granules
- zooglea
- biofilm
- activated sludge

Hans-Curt Flemming & Stefan Wuertz, 2019

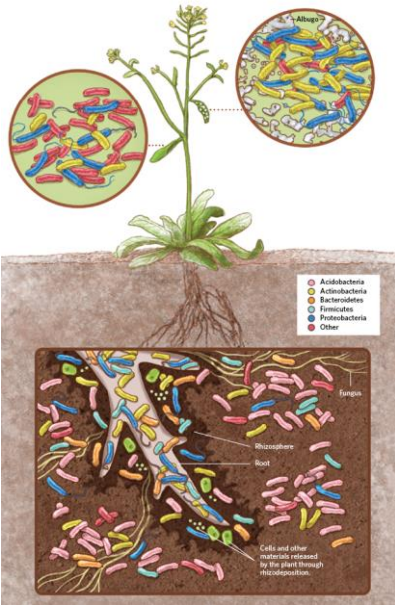
Microbial communities

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

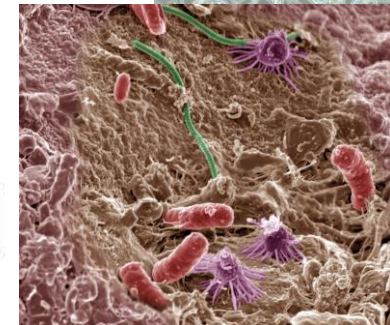
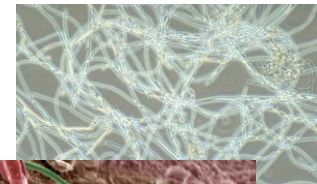
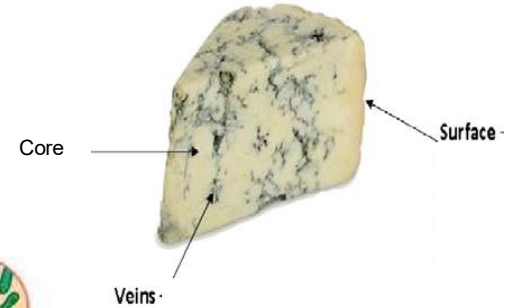
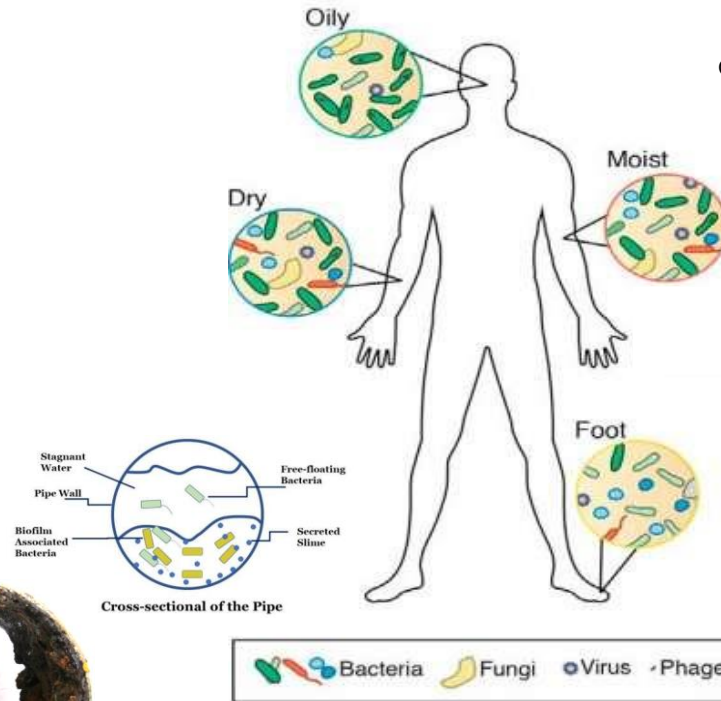
- Resource competition
 - Extracellular enzymes
- Metabolic interactions:
 - Co-metabolism
 - Cross-feeding
 - Sequential utilization (nitrification)
 - Chemical modification of environment (pH)
- Signaling
- Trophic level interactions
 - Parasitism
- Horizontal gene transfer
- Co-evolution



Examples of microbial communities



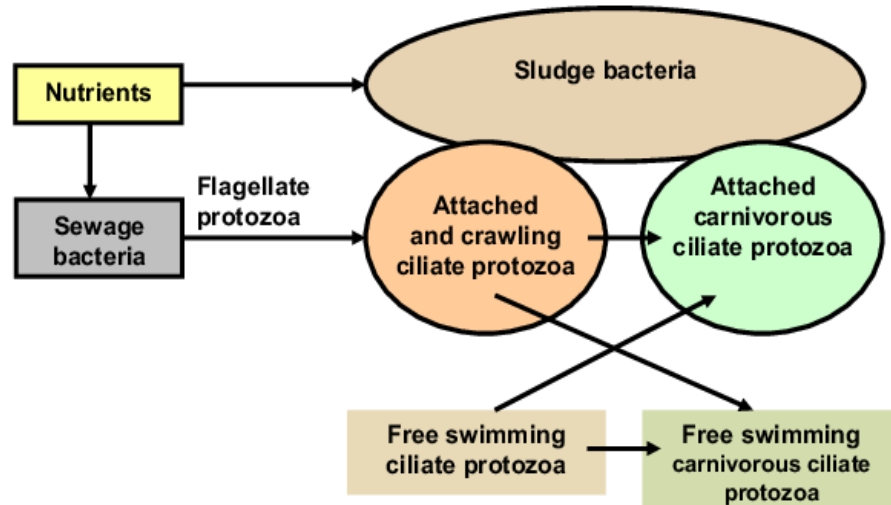
MESA SCHUMACHER



Activated Sludge

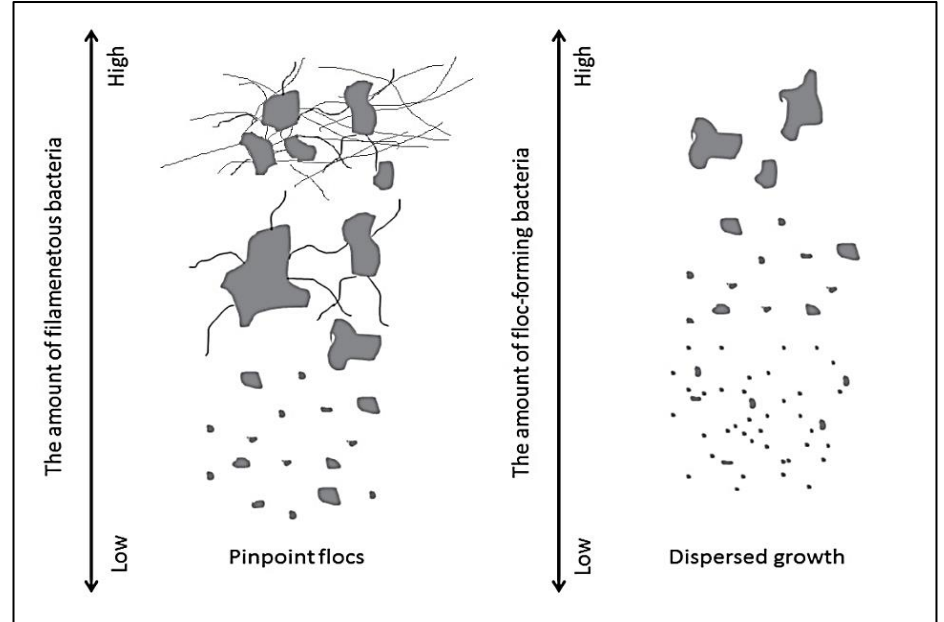
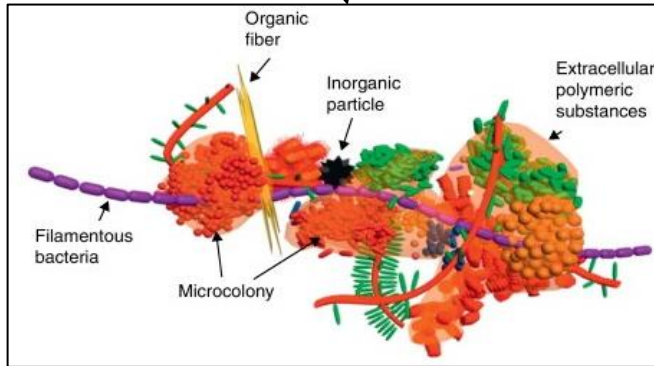
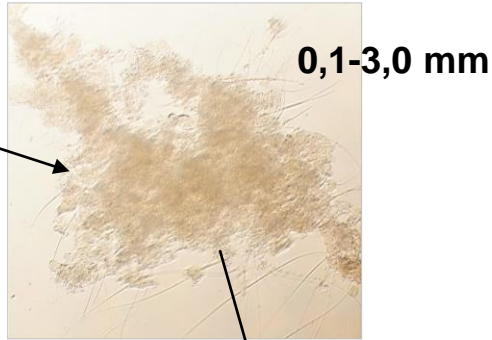
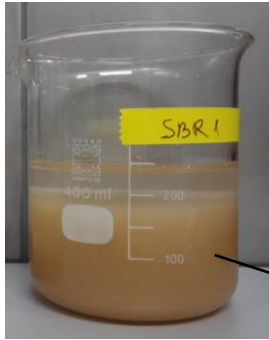
- community of microorganisms, metabolizing target organic pollutants in wastewater.

Microorganisms are aggregated into *flocs*



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

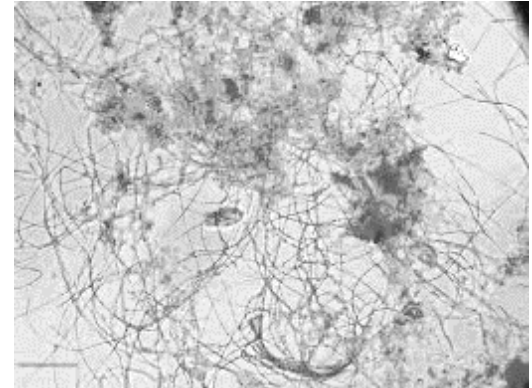
Floc formation



Bulking and foaming

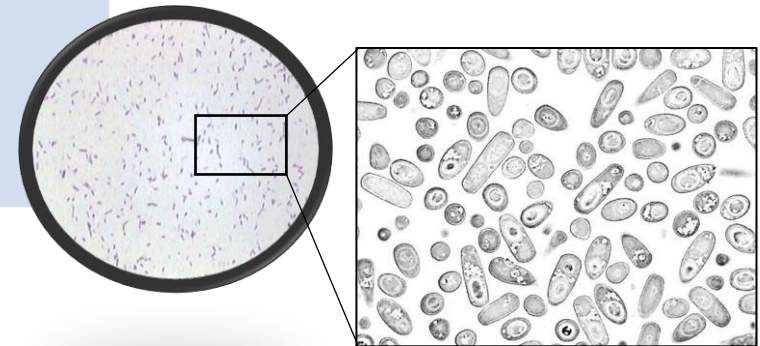
- can be caused by (excessive) formation of filamentous microorganisms
- normal flocs - a balance between floc-forming and filamentous microorganisms results in strong flocs that keep their integrity in the aeration basin and settle well in the sedimentation tank

Microscopy is an effective tool for monitoring of activated sludge conditions, which can be used to immediately diagnose the causes of treatment problems



Activated sludge bacterial community

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



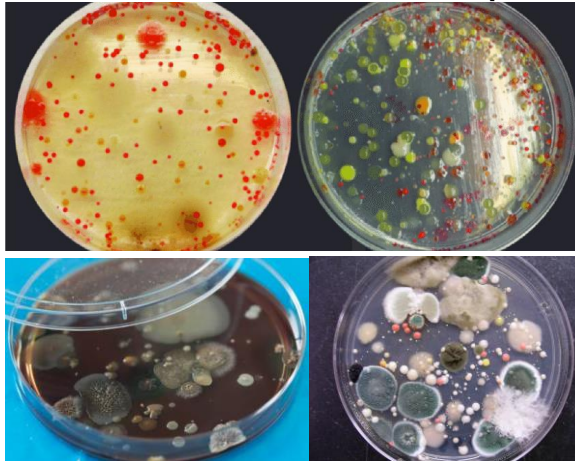
Identification of bacteria: morphological tests

- 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

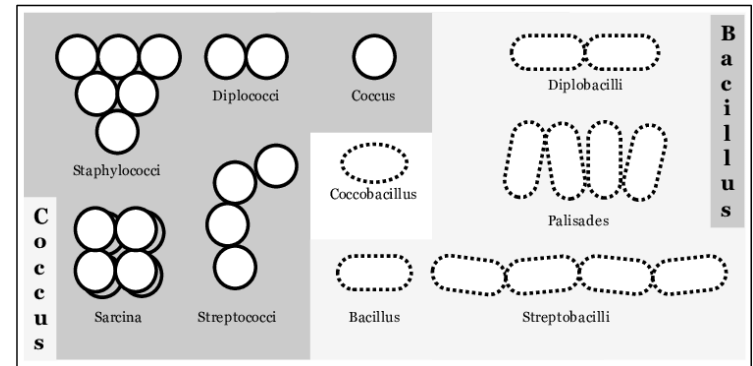
A colony is a visible mass of microorganisms on solid media all originating from a single mother cell

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

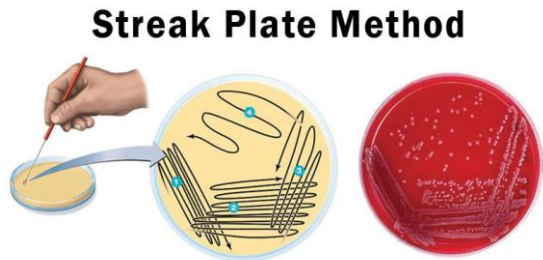
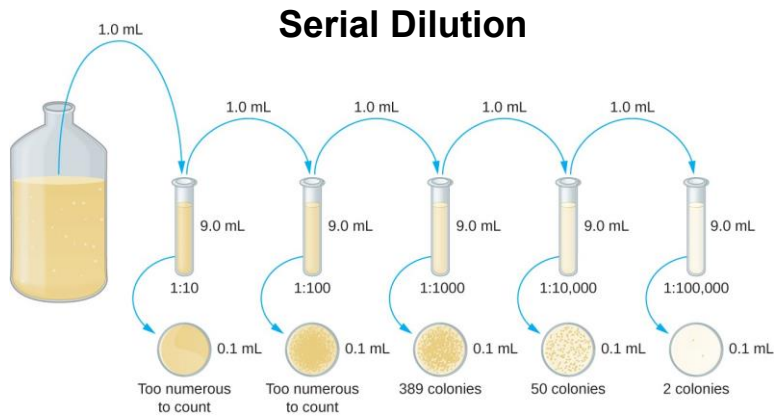
Colonies on Petri dishes/ culture plates



Shapes and arrangements



Plating methods



Pour Plate Method

- 1 Bacterial sample mixed with warm agar (45–50 °C)
- 2 Sample poured onto sterile plate
- 3 Sample swirled to mix, allowed to solidify
- 4 Plate incubated until bacterial colonies grow

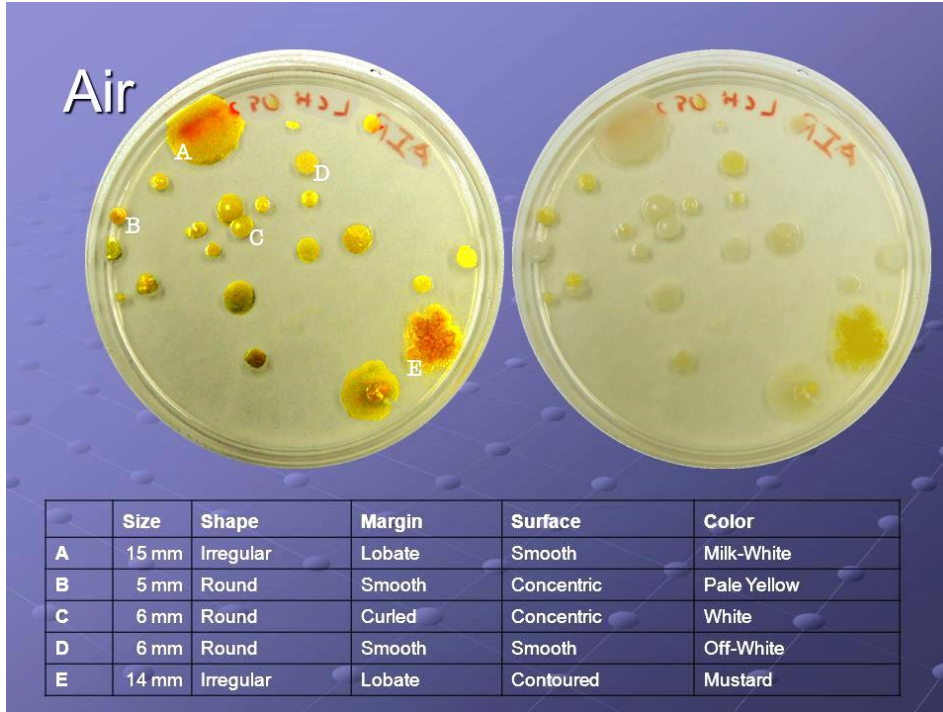


Spread Plate Method

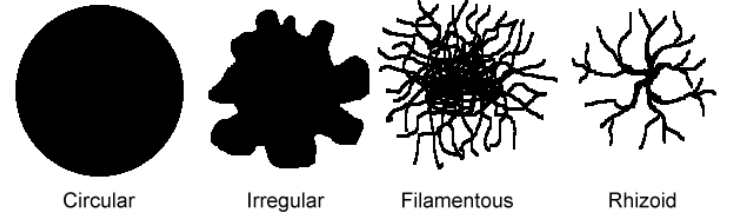
- 1 Sample (0.1 mL) poured onto solid medium
- 2 Spread sample evenly over the surface
- 3 Plate incubated until bacterial colonies grow on the surface of the medium



The Examination of Bacterial Colonies



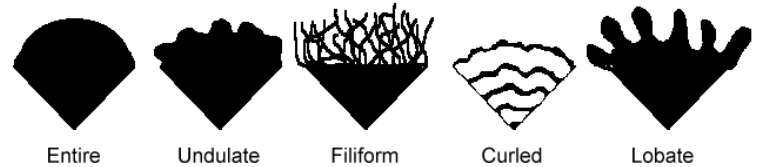
Form



Elevation

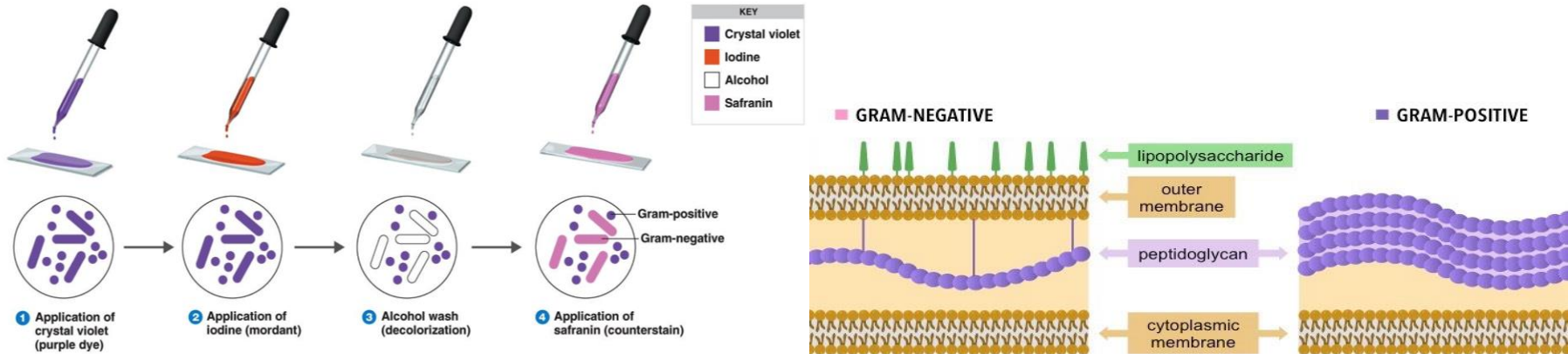


Margin



Identification of bacteria: biochemical tests

- cell wall structure



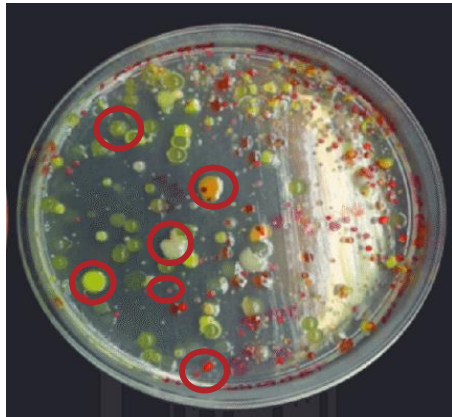
- more than sixty more tests

<http://www.microra.com/identify.htm>

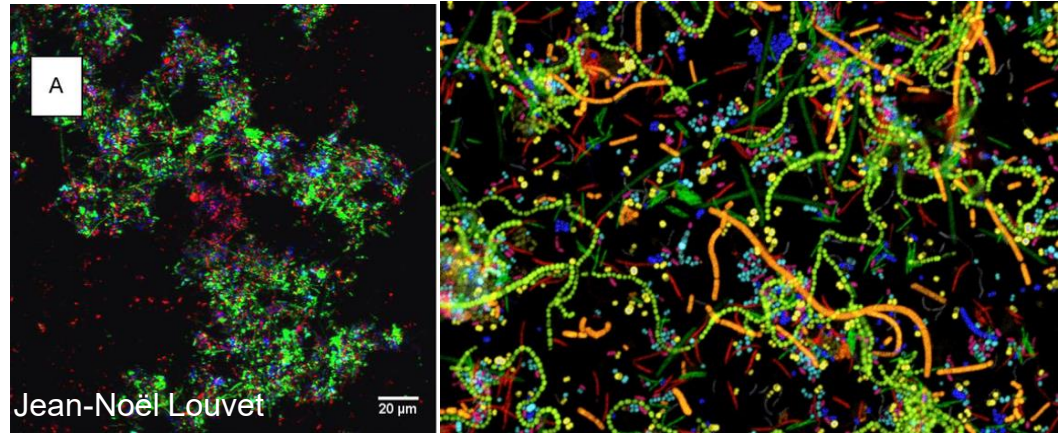
Unculturable bacteria

- 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



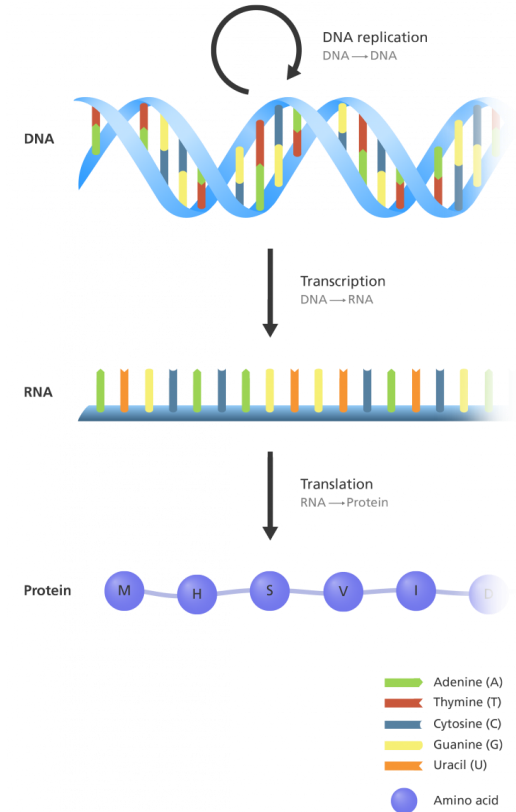
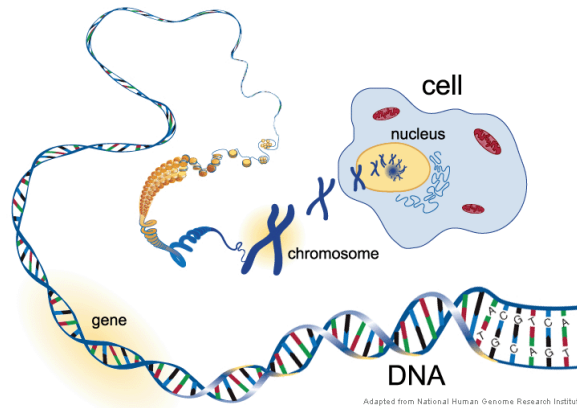
Activated sludge flocs



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



Why we need computers to answer microbiological questions?

- DNA contains information about all organisms existing
- Thousands of trillion pairs of bases (AT GC) from over 200 000 bacterial and archaeal species complete or draft genomes had been uploaded to public databases

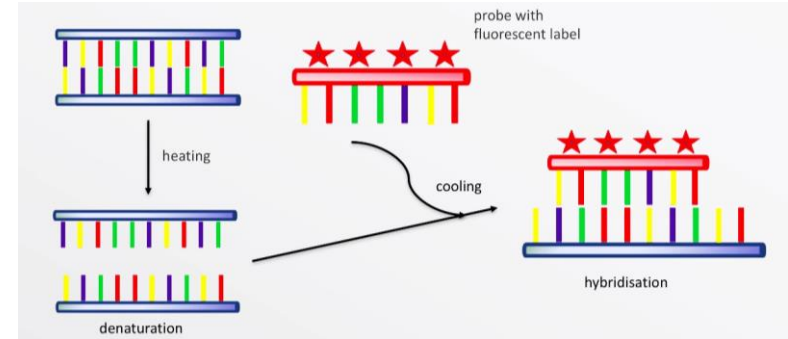
Just reading them would take more than 30 million years!



- ✓ algorithms
- ✓ software tools
- ✓ web servers (storage for big data)

Culture-independent methods

1. Fluorescent In Situ Hybridization (FISH)



2. 16S rRNA



Activated Sludge:

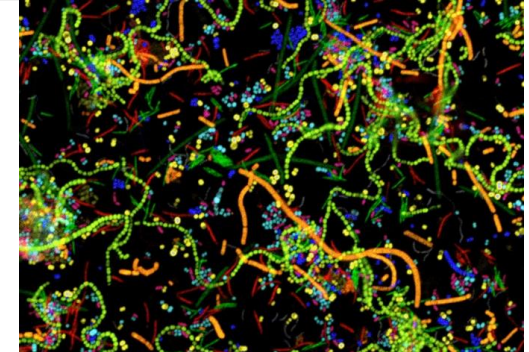
- Protozoa
- Bacteria
- archaea
- etc.



Total DNA

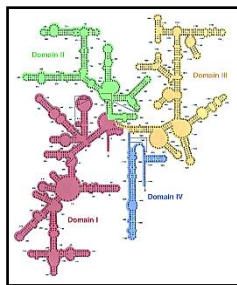


Prokaryotic
DNA

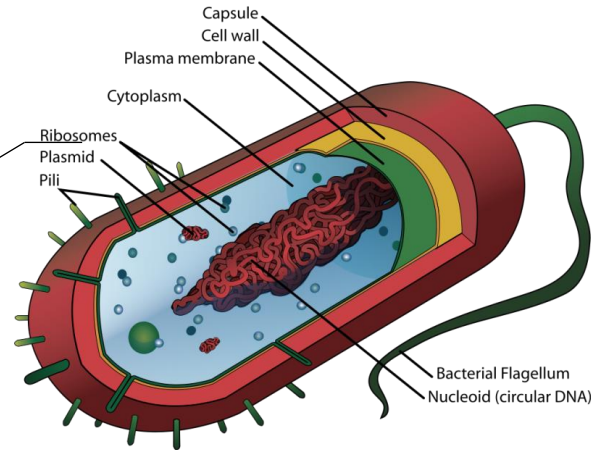
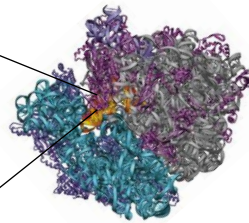


16S ribosomal RNA (16S rRNA)

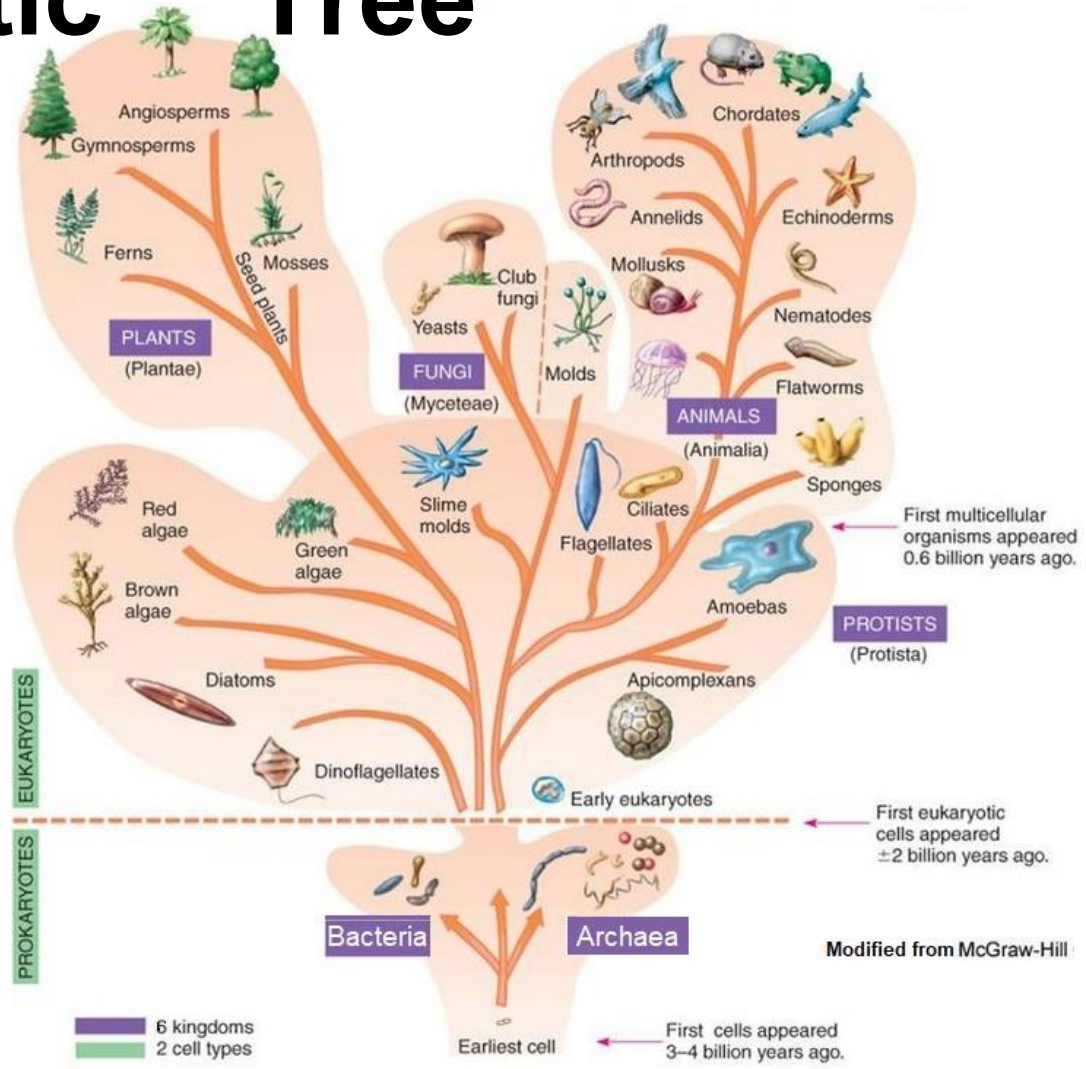
- has proved to be the most informative for investigating evolutionary relatedness
- used extensively in the classification and identification of *Bacteria* and *Archaea*
- ribosome – molecular structure (15000 in each *E. coli* cell , 10 mln in eucariotic cell)



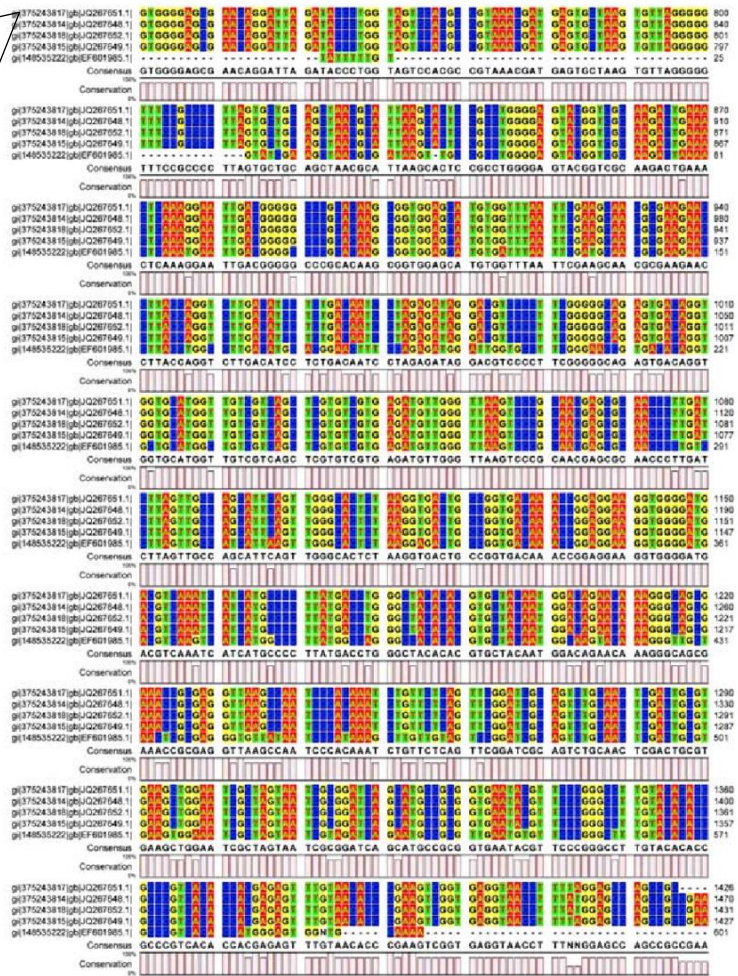
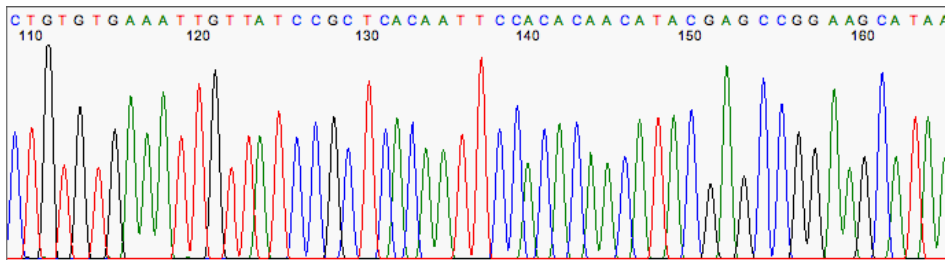
16S rRNA



Phylogenetic Tree



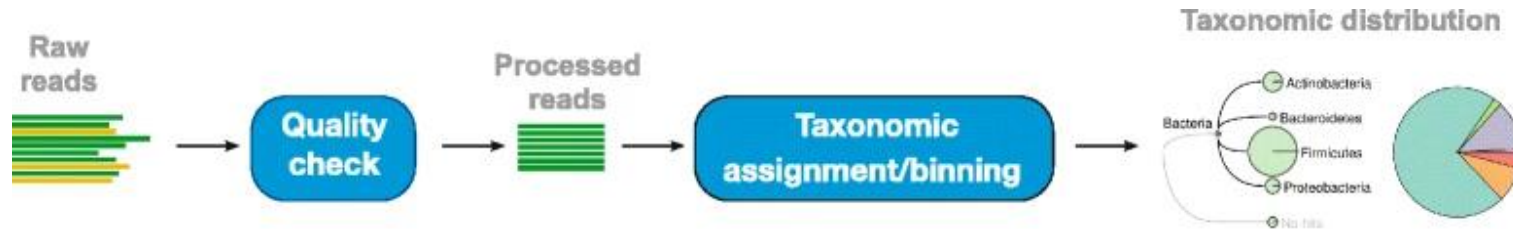
Sequencing



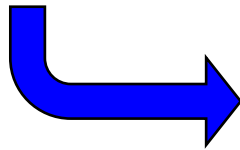
Metagenomics

– *microbial community genomics*

Total genome of all the organisms is taken directly from their natural environment



Similar sequence variants of the 16S rDNA are assigned as *Operational Taxonomic Units* (OTUs)

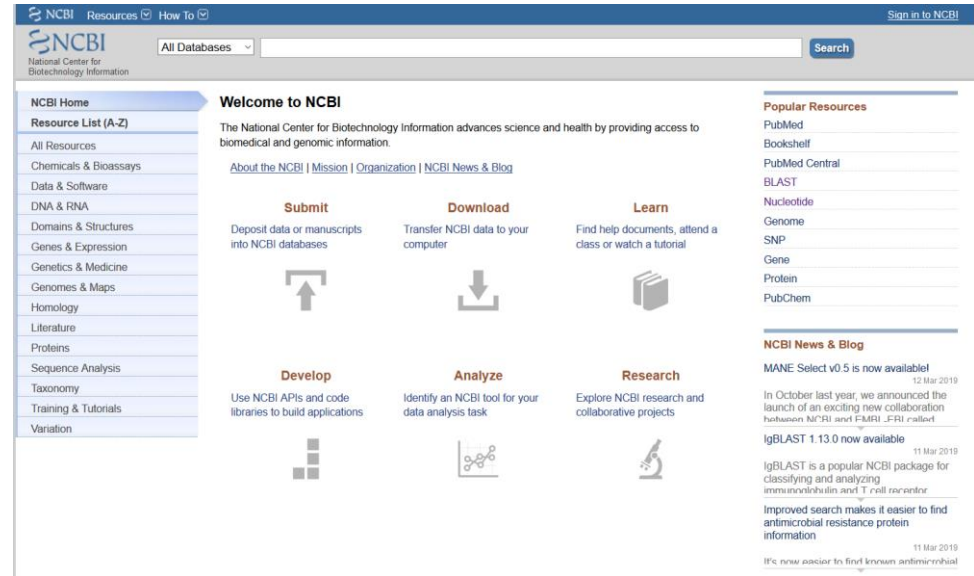


Defining microbial composition, presence and abundance (a sample can contain >10 000 species)

Taxonomic classification tools for 16S reads

Some examples of gene databases:

- SILVA
- RDP
- Greengenes
- EMBL
- **NCBI (GenBank)**



The screenshot shows the NCBI homepage with a navigation menu on the left, a search bar at the top, and a main content area with several service icons: Submit, Download, Learn, Develop, Analyze, and Research. The right sidebar contains 'Popular Resources' and 'NCBI News & Blog' sections.

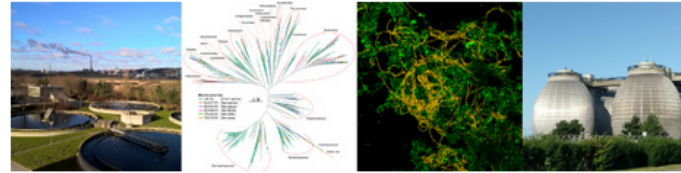
Basic Local Alignment Search Tool (BLAST) is a software for searching similarities in DNA sequences and/or amino acid (protein) sequences

MiDas database



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MiDAS: Field Guide to the Microbes of Activated Sludge and Anaerobic Digesters



The MiDAS (Microbial Database for Activated Sludge) field guide aims to summarize all the knowledge about the physiology and ecology of the important microorganisms present in engineered ecosystems of activated sludge plants, anaerobic digesters, and related wastewater treatment systems, ultimately creating a universal guide to the field.

Based on many years of collaboration with Danish wastewater treatment plants we developed the ecosystem-specific MiDAS taxonomy. It is a comprehensive, automated and curated taxonomy providing species-level resolution (Dueholm et al. 2020). In the near future, the taxonomy will be extended even further based on our recent [global campaign](#) in order to expose near-complete microbial diversity present in these ecosystems across the world.

Vision: MiDAS provides an ecosystem-specific taxonomy that together with the field guide links identity to function for the microbes in wastewater treatment and bioenergy systems.

The MiDAS taxonomy can be used to classify and provide placeholder names for unknown sequences at the species-level, and the online MiDAS field guide links the identity to a referenced summary of their in situ metabolism, morphotypes, and abundance in influent wastewater, activated sludge, and anaerobic digesters. Moreover, the BLAST function allows you to classify your sequences directly online.



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new [@WaterRes_J](#) paper by [@ChenjingJiang](#) and colleagues from [@PHNLab](#) explains how process parameters and environmental factors shape active fraction of microbial community in anaerobic digesters
[https://www.sciencedirect.com/science/article/pii/S0043135421000695 ...](https://www.sciencedirect.com/science/article/pii/S0043135421000695...)
Posted on Feb 19, 2021

Which PAO accumulate the most polyP? Check bioRxiv for "Quantification of biologically and chemically bound phosphorus in activated sludge from full-scale plants with biological P-removal" by Francesca Petriglieri and others from [@PHNLab](#)
[https://www.biorxiv.org/content/10.1101/2021.01.04.425262v1 ...](https://www.biorxiv.org/content/10.1101/2021.01.04.425262v1...)
Posted on Jan 12, 2021

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Practical exercise

OTU X

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