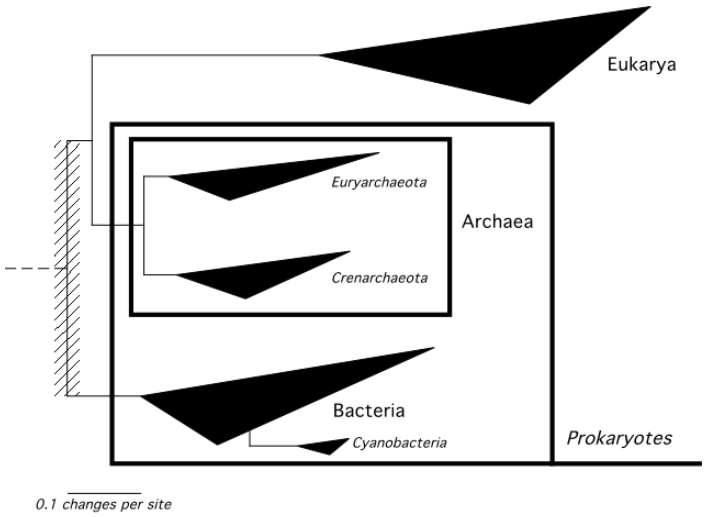
Microbial evolution and phylogeny



Evolution

Changes over time within the lineage of an organism that leads to the formation of novel species or to a variation within a species.

Evolution of life on Earth
Geological und fossile evidences

Million years ago

4600 Formation of planet Earth

3500 Microbial life (stromatolites)

2800 O_2-producing photosynthesis by *Cyanobacteria*

2000-1800 Accumulation of O_2 in the atmosphere

Early microorganisms
• have developed app. 3.6 to 4.0 billion years ago

Metabolism
• ability to collect nutrients to transform them and to gain energy from it

Reproduction
• ability to replicate own attributes and to transfer them to offsprings

Environmental conditions on early Earth

- reducing atmosphere. No oxygen (O_2)
- important compounds: H_2O, CH_4, CO_2, N_2, NH_3, CO, H_2, H_2S
- surface temperature: partly more than 100°C
- strong UV radiation, electric discharge

Miller-(Urey) Experiment

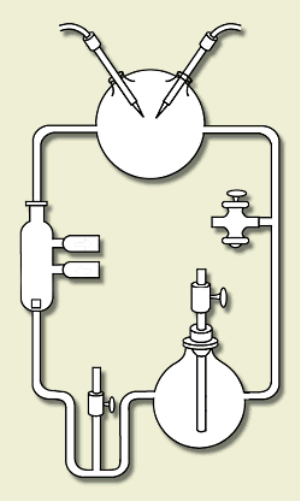
- simulation of early earth conditions in the laboratory
- leads to the formation of biochemical relevant molecules:

  sugars, amino acids, purines & pyrimidines, nucleotides, thioester, fatty acids
- accumulation of these compounds due to a lack of biological degradation
- after cooling of earth: stabilisation of the organic compounds and inclusion in membrane-like structures

→ **Formation of cell-like structures**

The Miller-Urey experiment

Principle of the experiment



Two main reaction chambers

Water is circulating through the apparatus

Lower vessel:
simulation of the hot paleo-ocean

Upper vessel:
simulation of the paleo atmosphere:
H_2, NH_3, methane and steam

lightning simulated by electrodes

Reaction products are led through a **condensor**

W-formed construction:
capture of water soluble reaction compounds at the bottom of the apparatus

Miller-Urey experiment

Stanley L. Miller and his appatatus

The products of the experiment

Tar	85 %
div. Carbolic acids	13.0 %
Glycin	1.05%
Alanin	0.85%
Glutamic acid	traces
Asparagic acid	traces
Valin	traces
Leucin	traces
Serin	traces
Prolin	traces
Threonin	traces

In 1969, a meteorite was found in Australia was showing the same composition of amino acids as the in the Miller-Urey experiment!

Building blocks of life in stellar dust and gas clouds?

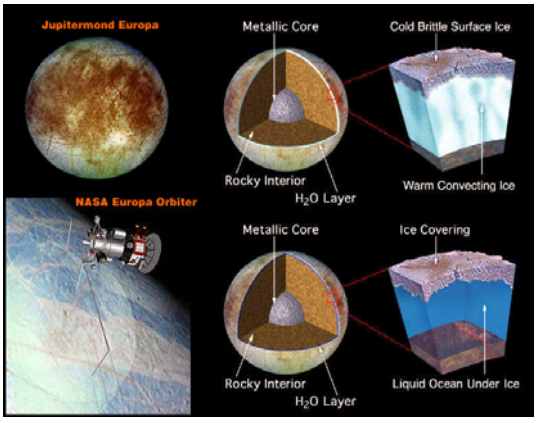
- Simulation of environmental conditions in vacuum chambers (Uni Bremen)
  - icy aluminum plate
  - vaporization of simple chemical compounds
  - H_2O, CO_2, NH_4, CH_3OH get attached
  - radiation simulated by a strong UV-lamp
  - formation of complex organic compounds
  - detection with an Infrared-spectrometer

After heating up of the aluminum block:

Detection of Sixteen different amino acids within the icy-layers

Habitability on Europa

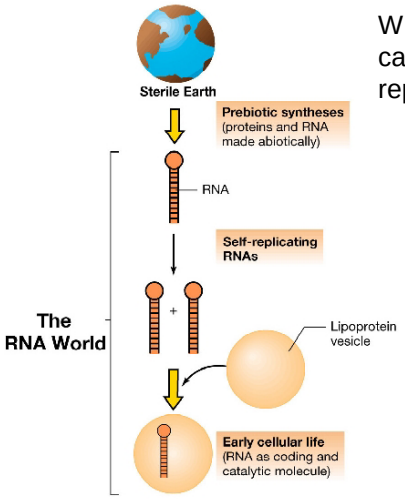
- Thickness of the icy and dusty shield: more than 80 to 170 km
- The proposed ocean has a thickness of more than 100 km
- Regeneration every 10 million years (melting of the lowest layers)
  - Gravity forces of Jupiter is dispersing the surface of Europa, meltwater is flowing upwards through cracks in the ice



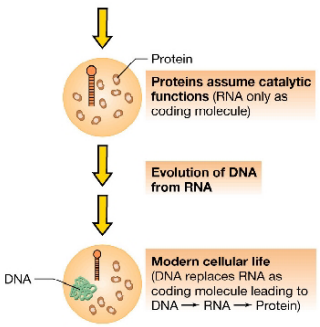
Back to Earth

Open question: How was the first organism formed?

The RNA world: Possible scenario for the evolution of cellular life



With time, proteins replaced the catalytic functions of RNA and DNA replaced the coding functions of RNA



Self-replicating RNAs could have become cellular entities by becoming stably integrated into lipoprotein vesicles.

Metabolism:
• must have been **anaerobically** (no O_2 in the atmosphere)

Energy yield:
• oxidation of organic compounds **(chemoorganotrophy)**
• oxidation of inorganic compounds **(chemolithotrophy)**
• (driven by light **phototrophy)**

Metabolic pathway must have been simple

e.g. formation of iron sulfide

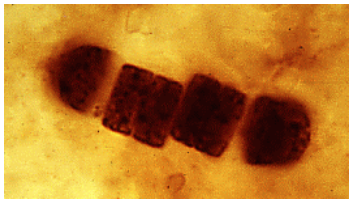
$FeCO_3 + 2\ H_2S \rightleftharpoons FeS_2 + H_2 + H_2O + CO_2$

$FeS + H_2S \rightleftharpoons FeS_2 + H_2$

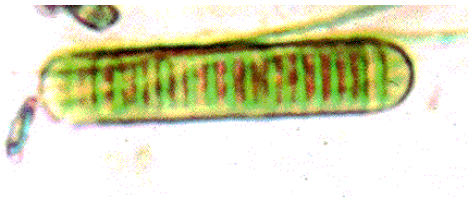
only a few enzymes nessecary!

Fossile evidences for microbial life

Cyanobacteriea from the Precambrian
(app. 3.5 billion years old), oldest known fossiles



fossile Cyanobacterium from North-Australia
(app. 1 billion years old)



actual living Cyanobacterium (*Oscillatoria*)

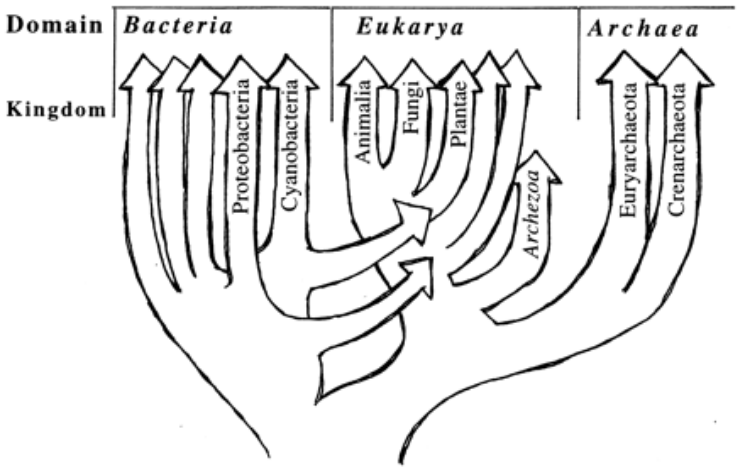
Stromatolites

Cyanobacteria can form **Stromatolites**

Laminated structure, embeddind in sediments;
Bacteria produce calcium carbonates
Thin-sections show fossile cyanobacteria and algae

Phylogeny:

Classification of species in superior taxa and construction of phylogenetic trees based on evolutionary relationships.



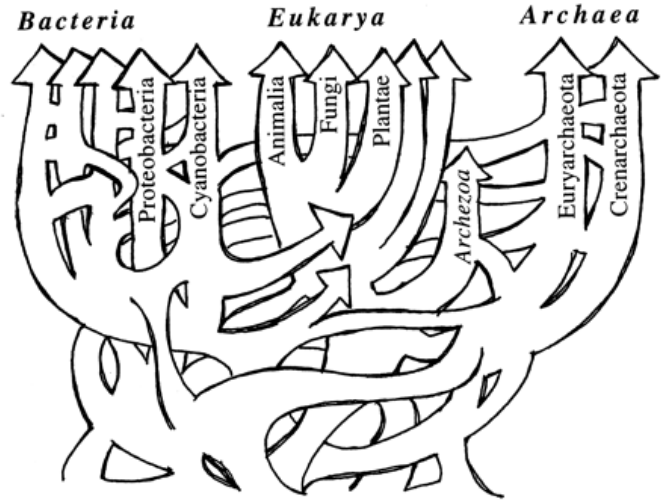
Endosymbiotic theory:
Proteobacteria ⇒ Mitochondria
Cyanobacteria ⇒ Chloroplasts

Novel theory: There was not **the** common ancestor
Life has evolved out of multiple ancestoral cells.

Some have prevailed to become ancestors of Bacteria, Archaea and Eukarya.

Horizontal gene transfer
- between organisms (even from different domains) might have played an important role evolution.

„Darwinian threshold“
- in the beginning: Horizontal gene transfer (open systems of cells)
- afterwards: Etablishment of cell compartments (Horizontal gene transfer less important)



Variety of ancestoral cells
Horizontal gene transfer between organisms

How many different bacteria do we expect?

Validly described species:

5 000 Prokaryotes (Bacteria und Archaea)
1 700 000 Eukaryotes

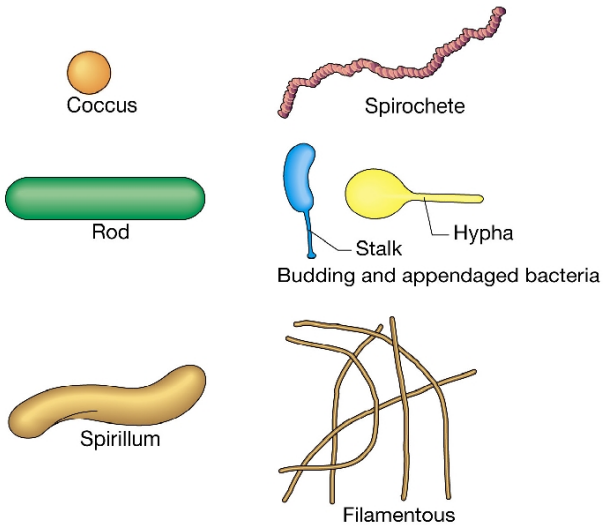
Estimations for different bacterial species in 30 g forrest soil

3 000 (Torsvik et al., 1990)
500 000 (Dykhuizen 1998)
(based on the same data set)

The big debate:
What is a species???

How to classify a microbe?

Microbial morphology is limited



only a weak hint to determine microbial affiliation

Identification of a microbe by classical microbiological methods

Isolation from mammal

gram staining

gram negative — gram positive

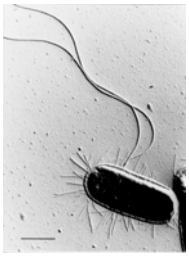
rod shaped — not rod shaped

fakultative aerob — obligate aerob

fermentation of lactose, production of acid and gas — no fermentation of lactose

further metabolic tests

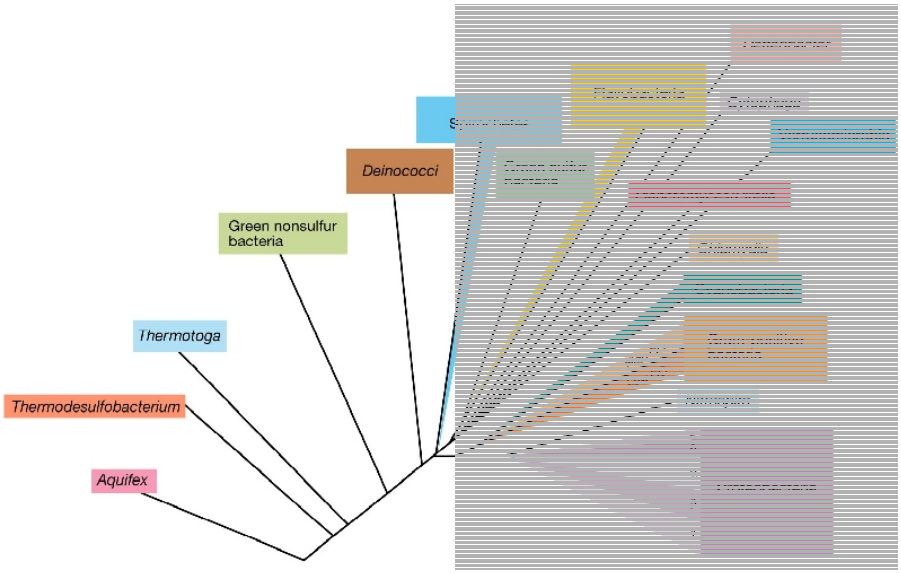
Identification as *Escherichia coli*



Hierarchical structure in taxonomy

Bacteria	domain
Proteobacteria	phylum
Gammaproteobacteria	class
Enterobacteriales	order
Enterobacteriaceae	family
Escherichia	genus
Escherichia coli	species
Escherichia coli K12	strain

Phylogenetic overview on the bacterial domain



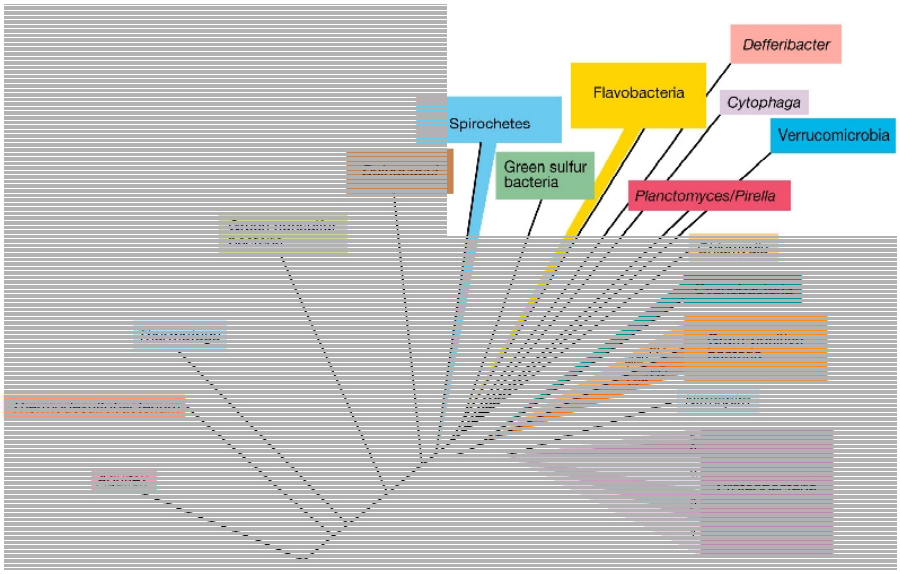
Aquifex-Hydrogenobacter **group**
- hyperthermophile (opt. >80°C), chemolithotroph,
- *Aquifex* probably most similar to bacterial ancestor

Thermotoga
- hyperthermophile, chemoorganotroph

Green non sulfur bacteria (GNSB), ***Chloroflexi***
- partly phototroph, thermophile (opt. 45-80°C),
- chemoorganotroph

Deinococcus **group**
- partly radiation resistant (UV- and gamma ray)
- (*D.radiodurans* extremly effective DNA repair mechanisms),
- partly thermophile

Phylogenetic overview on the bacterial domain



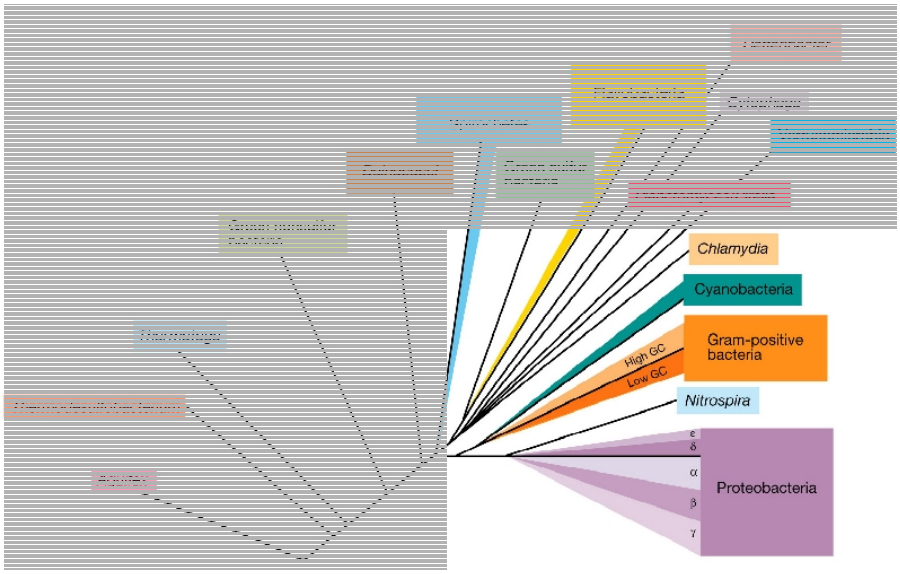
Spirochetes
- conspicious morphology, special apparatus of movement, partly pathogen

Gree sulfur bacteria
- strictly anaerobic, obligat phototroph,
- can utilise simple organic compounds, if there are reduced sulfur compounds available

Cytophaga Flavobacteria Bacteroidetes (CFB)
- aerobic and anaerobic, polymer degrader
- some show gliding movement

Planctomyces
- reproduction by budding, no peptidoglycan
- aerobic, mainly aquatic

Phylogenetic overview on the bacterial domain



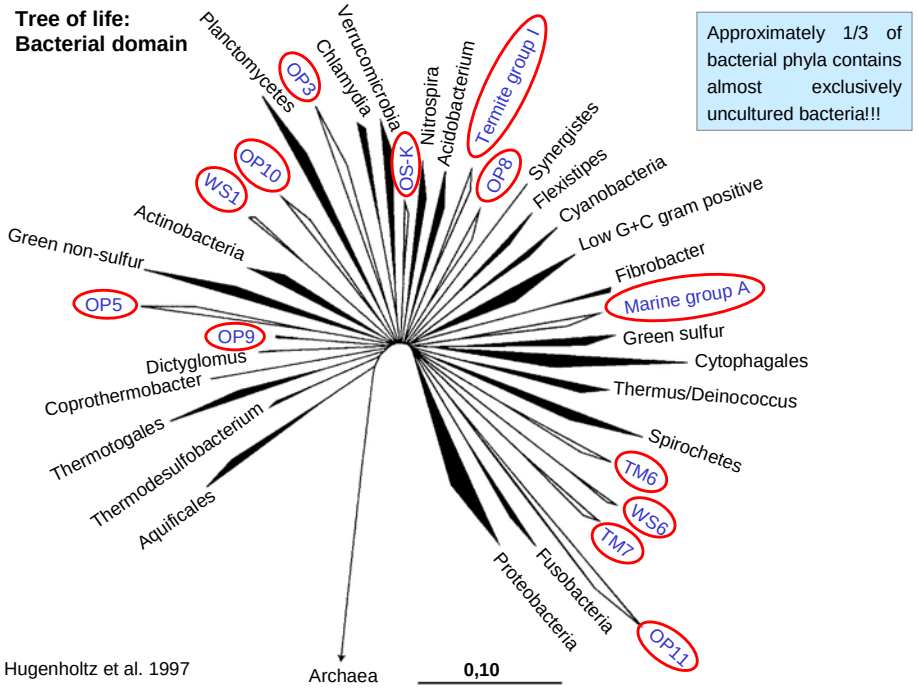
Chlamydia
• obligate intracellular parasites, many pathogens

Cyanobacteria
• oxygenic phototrophs

Gram positives (*Firmicutes*)
• big heterogeneous group divided into two subgroups
• high GC (*Actinobacteria*) and low GC gram positives

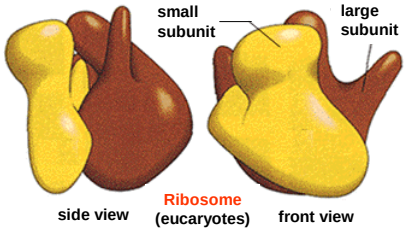
Proteobacteria
• biggest group, pysiologically diverse
• five subgroups (alpha, beta, gamma, delta, epsilon)

Molecular techniques



Analysis of ribosomal nucleic acids

• Ribosomes are cellular maschines for the construction of proteins and enzymes



- present in all living organisms
- high copy number
- up to 20.000 ribosomes per cell

The ribosomal RNA is the backbone of the ribosome

- sufficient number of nucleotides for phylogenetic analyses
- 16S rRNA: app. 1.500 bp

Ribosomal RNA for phylogenetic analyses

Due to the essential function of ribosomal nucleic acids:

- Mutation is often lethal
- Independent (constant) pressure of selection
- Highly conserved at many positions
- Comparison of analogous, but variable sequences
- Almost no gene transfer

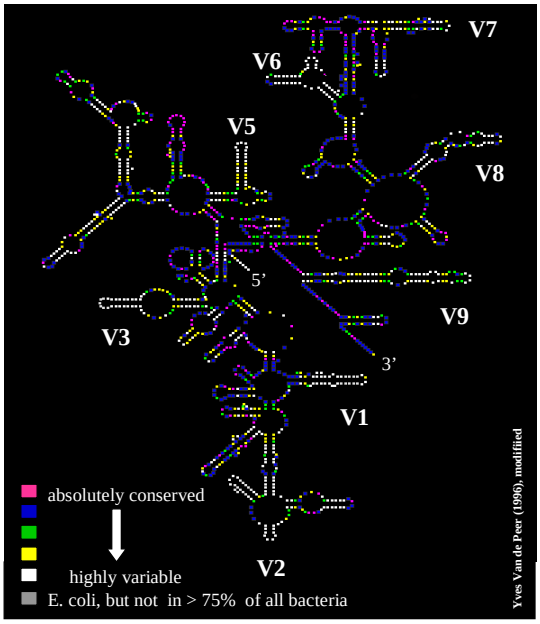
Changes of sequences happen with a constant speed, but slowly enough to mirror the whole time of bacterial evolution (Carl Woese, 1987)

The evolution of the molecule mirrors the evolution of its host („molecular clock“)

The prokaryotic 16S rRNA

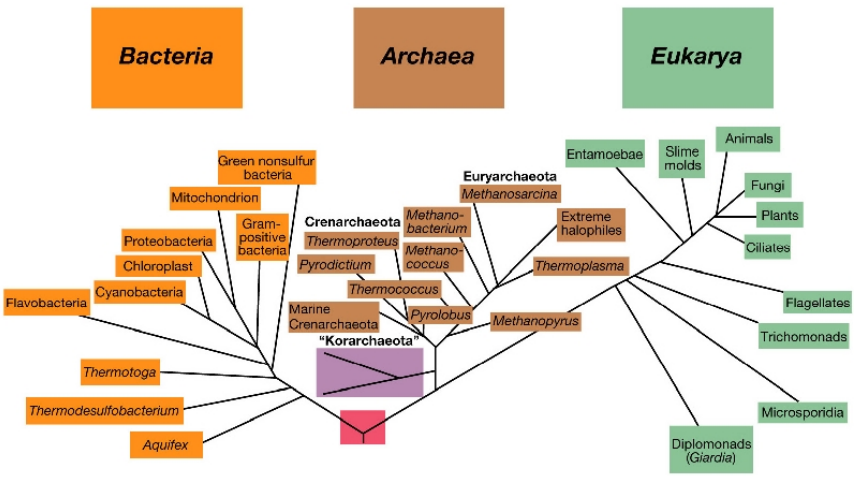
• The molecular clock shows a different speed in some areas of the rRNA.

• Mutations in highly conserved regions happened evolutionary at earlier stages than in variable regions.

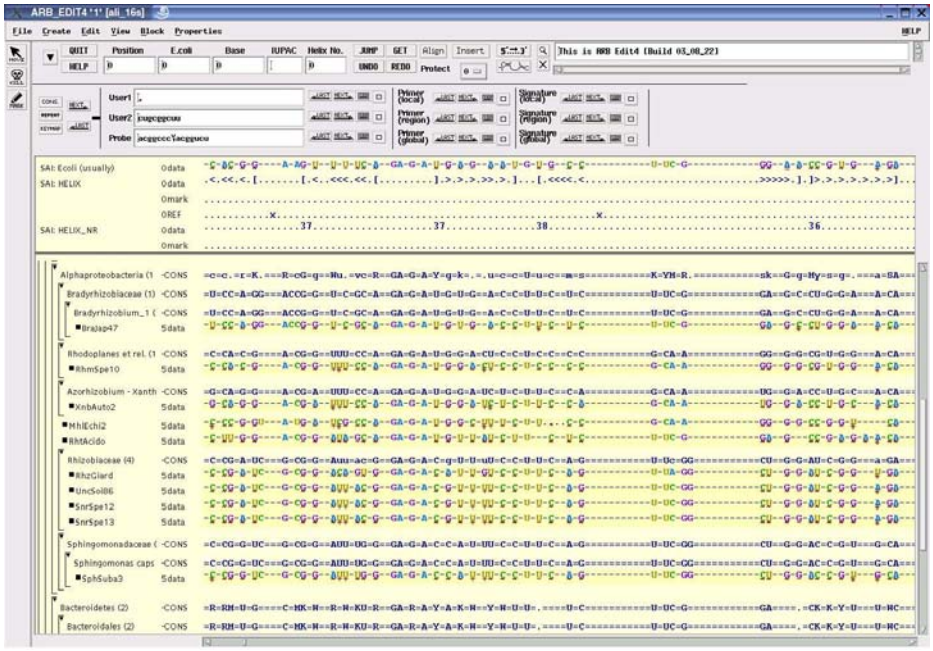


The 16S rRNA as a „molecular clock“ of evolution

The investigation of phylogenetic relationships according to rRNA-sequences by Woese & Fox (1977) finally led to the classification of all organisms into the domains: Bacteria, Archaea and Eukarya (Woese, 1990).



Alignment of 16S rRNA sequences



16S rRNAs in the database

Currently 1 074 075 sequences

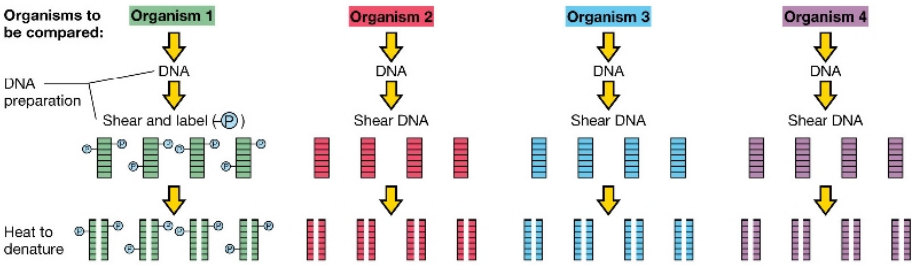
National Center for Biotechnology Information

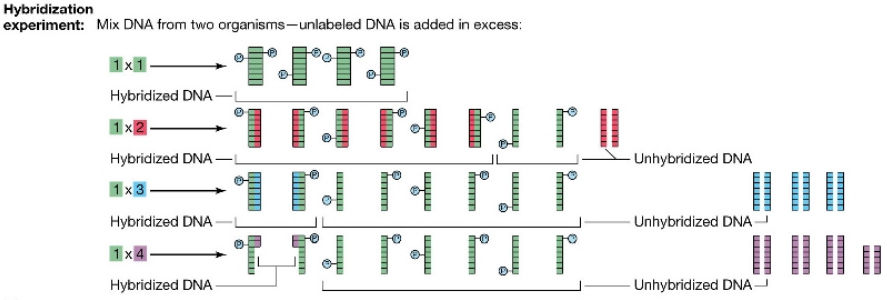
National Library of Medicine National Institutes of Health

Aug 31, 2009

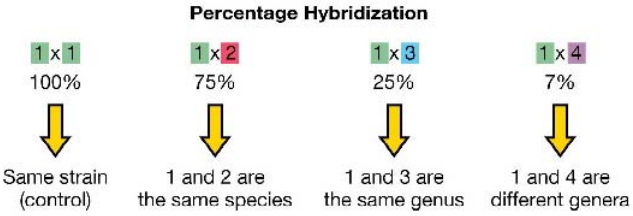
Is it enough to define a species?

DNA DNA hybridization

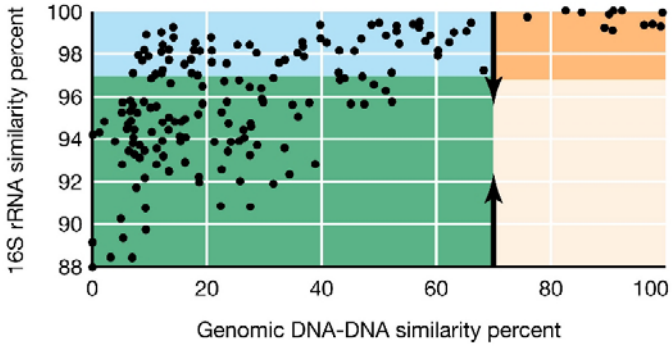




Results and interpretation



The species level



Application of molecular probes

- **Hybridization**
  - Probe (Oligonucleotide) at a target sequence (mostly 16S rRNA)
- **Specificity**
  - Strain, family, ... up to the domain (dependent on target sequence)
- **Most important technique**
  - **Fluorescence-In-Situ-Hybridization, FISH**
    - with fixed cells (binding at ribosomes)
      - signal enhancement by higher ribosome content or
      - enzymatic amplification (CARD-FISH)

Specific detection microorganisms

Fluorescence In-situ Hybridisation, FISH:

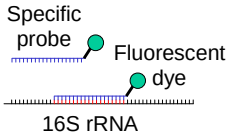
Cells fixed on filter

Microbial community

Hybridisation:

Probe binds at a target sequence (mostly 16S rRNA)

Signal enhancement by higher ribosome content

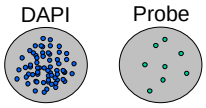


Specificity:

Strain, family, ... up to domain

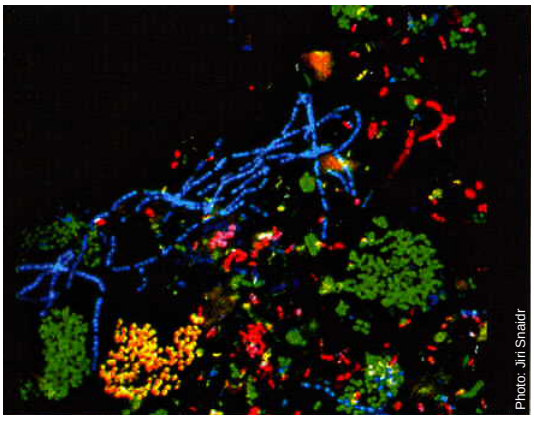
Quantification:

Non specific vs. specific signals

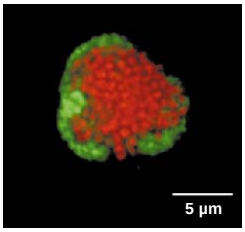


Analysis of bacterial communities by Fluorescence-In-Situ-Hybridization, FISH

- Coupling of molecular “probes“ with fluorescent dyes
- Annealing at speciffic regions of the rRNA
- Staining of cells on different phylogenetic levels
- Detection under a microscopic slide (in situ)



Anaerobic methane oxidsing consortia



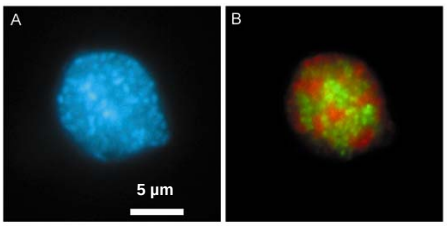
ANME2 (EelMS932)

Desulfosarcina (DSS658)

Boetius, et al. (2000)
Nature. 407:623-626

detected in gas hydrate
bearing sediments

DAPI **CARD-FISH**



detected in
tidal flat sediments

Archaea (ARCH915)

Desulfosarcina (DSS658)

Stronghold of Fluorescence-In-Situ-Hybridization is the MPI in Bremen!

Questions:

Is there a 16S rDNA?
Why do we prefer to analyse DNA?
When do we analyse RNA?
How can we analyse strains below the species level?
What will future bring?