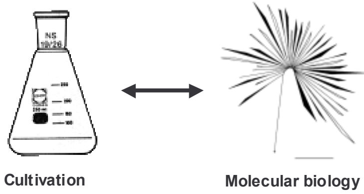
Analysis of microbial communities by cultivation and molecular biological techniques



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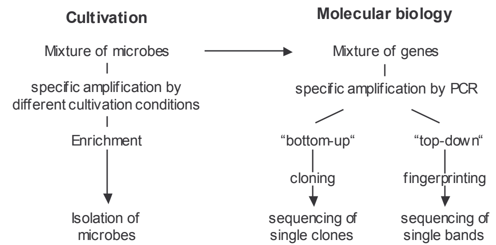
One of the main tasks in both approaches is to sort out single parts of the puzzle in order to get a more detailed view on microbial communities



Why both approaches?

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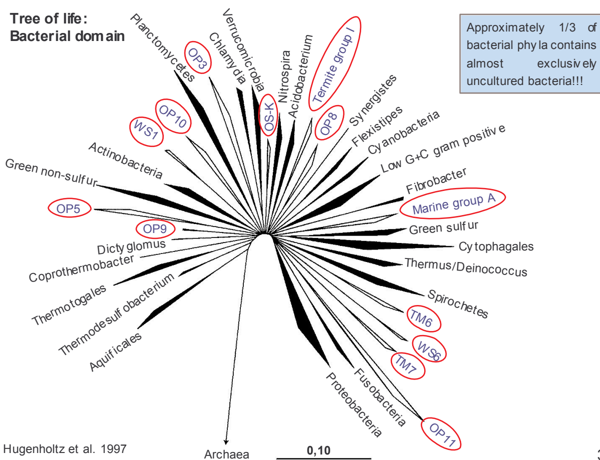
Tree of life: Bacterial domain



Approximately 1/3 of bacterial phyla contains almost exclusively uncultured bacteria!!!

Hugenholtz et al. 1997

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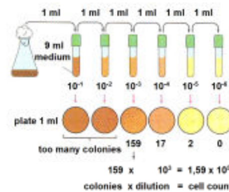
Cultivation dependent techniques

- **Advantage**
  - Investigations of microbial physiology, gene-regulation or production of metabolites
- **Disadvantage**
  - Many bacteria are not easy to cultivate
  - Extreme time consuming (several months to years)



- MPN-technique (Most Probable Number) dilution series in 3-5 parallels
- plating or liquid cultures

less than 1% of microbial strains from a given sample can currently be cultivated

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Molecular biological techniques

- **Advantage**
  - No cultivation steps
  - Non-cultivated organisms are included
- **Disadvantage**
  - Further investigations are often impossible

Information			**No Information**
16S rDNA:	Who is there or was there?	→	What happens?
16S rRNA:	Who is active or alive?		
Functional genes:	What happens?	→	Who?

Molecular biological techniques?

... back to the textbook

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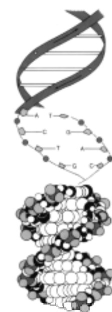
The DNA as a carrier of genetic information

MOLECULAR STRUCTURE OF NUCLEIC ACIDS
A Structure for Desoxyribose Nucleic Acid
J. D. WATSON F. H. C. CRICK
Nature, VOL 171, page737, 1953



"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

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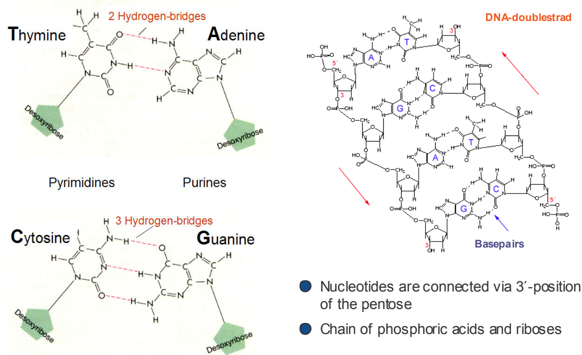
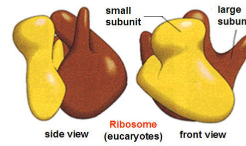
Construction scheme of the DNA



- Nucleotides are connected via 3´-position of the pentose
- Chain of phosphoric acids and riboses

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

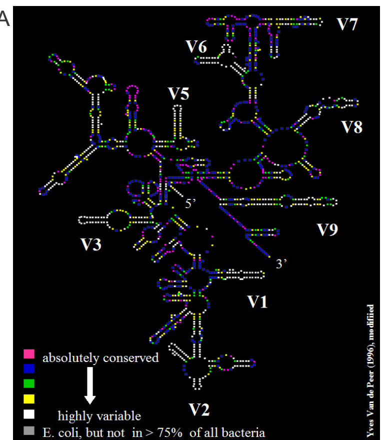
- **Due to the essential function of ribosomal nucleic acids:**
  - Mutation is often lethal
  - Independent (constant) pressure of selection
  - Highly conserved at many positions
  - Comparison of analogous, but variable sequences
  - Almost no gene transfer

- 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“)

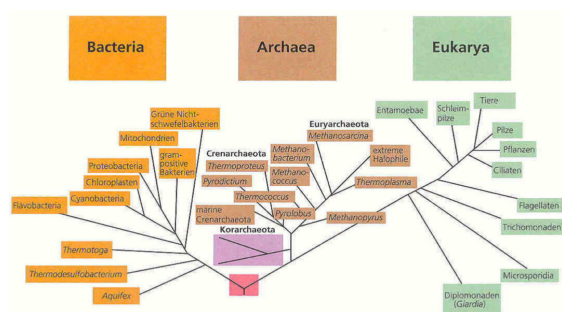
The prokaryotic 16S rRNA

- 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).



Staining of cells by DNA-dyes

Example: Acridine orange

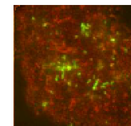
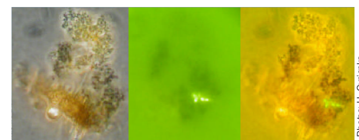


Photo: A. Batzke

Bacteria within a sediment-particle

Other DNA-dyes:
- DAPI, SYBRgreen


Photos: H. Cypionka

Iron sulfide particle phase contrast | fluorescent cells | combination of phase contrast and fluorescence

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)

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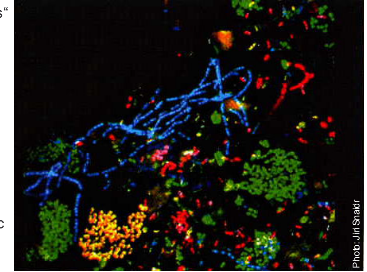
Analysis of bacterial communities by Fluorescence-In-Situ-Hybridization, FISH

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



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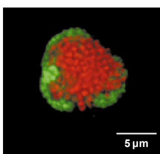
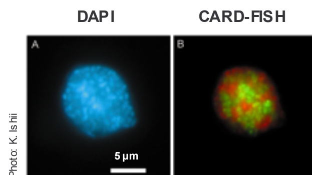
Anaerobic methane oxidsing consortia



ANME2 (EelMS932)
Desulfosarcina (DSS658)

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

detected in gas hydrate bearing sediments



detected in tidal flat sediments

Archaea (ARCH915)
Desulfosarcina (DSS658)

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

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Extraction of DNA and RNA from cells or environmental samples

- **Cell disruption**
  - **mechanically** freeze thaw, bead beater, ultra sonication
  - **chemically** detergences (SDS)
  - **enzymatically** lysozyme
- **Purification**
  - phenol / chloroform extraction
  - binding at matrices (glasmilk, silica) → many kits available!
  - precipitation with salt and alcohol

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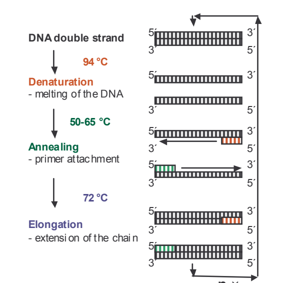
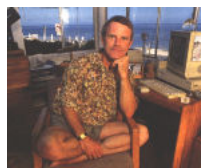
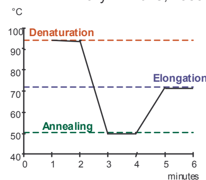
Principle of the Polymerase Chain Reaction



primers are "molecular biological tweezers"



Kary B. Mullis, 1983



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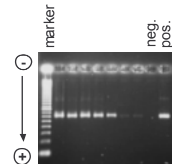
Electrophoresis of PCR products

Separation according to their charge (size)

DNA has a negative charge at neutral pH (phosphate groups)

Loading of PCR products



result of an agarose gel electrophoresis

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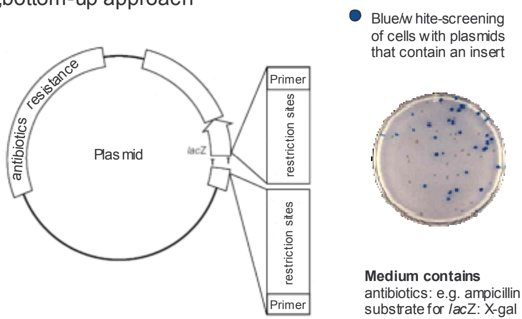
Isolation of sequences by cloning

„bottom-up approach“



- Blue/white-screening of cells with plasmids that contain an insert

Medium contains
antibiotics: e.g. ampicillin
substrate for *lacZ*: X-gal

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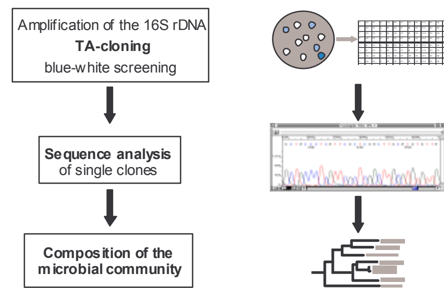
Analysis of a clone bank



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Phylogenetic “fingerprints“

“top-down approach“

- **The application of a pattern comparison has advantages at:**
  - high number of samples
  - differentiation of complex habitats
  - analysis of successions

- **Sequence specific separation: DGGE** (**D**enaturing **G**radient **G**el **E**lectrophoresis)
  - separation in denaturing gels

    according to the melting behavior of PCR products, **chemical gradient**

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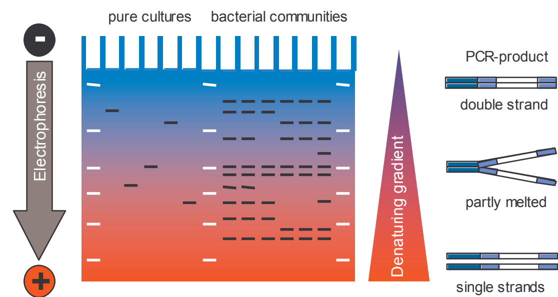
Melting behavior of PCR products and Scheme of a DGGE run



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Analysis of bacterial communities in tidal flat sediments

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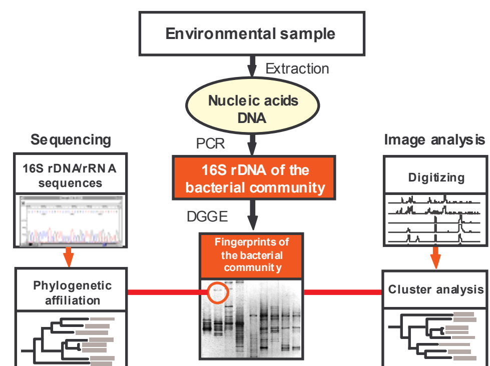
Fingerprinting of microbial communities



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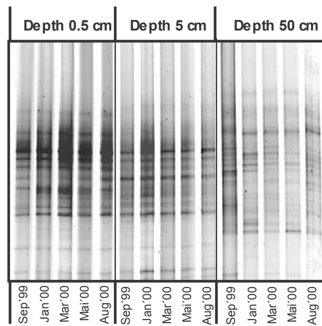
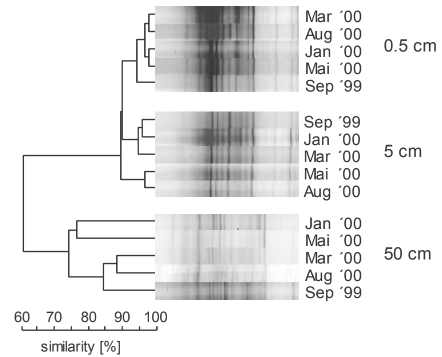
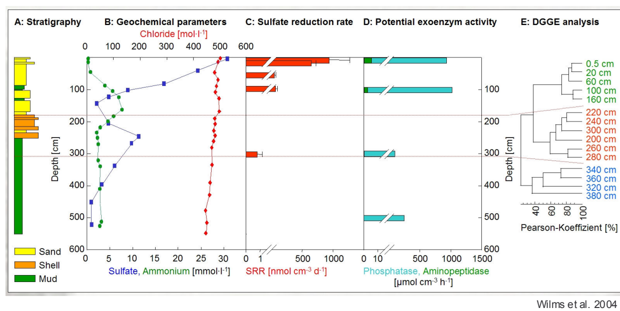
DGGE patterns of bacterial communities from tidal flat sediments



Cluster analysis of DGGE patterns

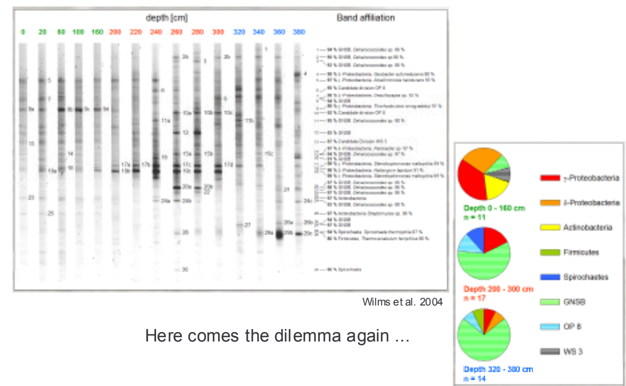


Combination with other data



Wilms et al. 2004

Detailed analysis of DGGE patterns



Here comes the dilemma again …

Finally: Why are we here?

Especially the deep biosphere is hardly explored.
So far we have only a limited number of isolates to analyze.
Many physiological processes are not fully understood.

Because of the hydrothermal flow, we have the chance to analyse microbial communities at higher temperatures and higher nutrient and energy supplies.

This makes it easier for us to cultivate microorganisms from the deep biosphere.

A spot like this provides us with the opportunity to analyse a highly active microbial community.

Take home message

Molecular biological analyses should always be accompanied by cultivation dependant techniques!

Thanks

Danke Merci (Tack)

ありがとう

Takk Gracias