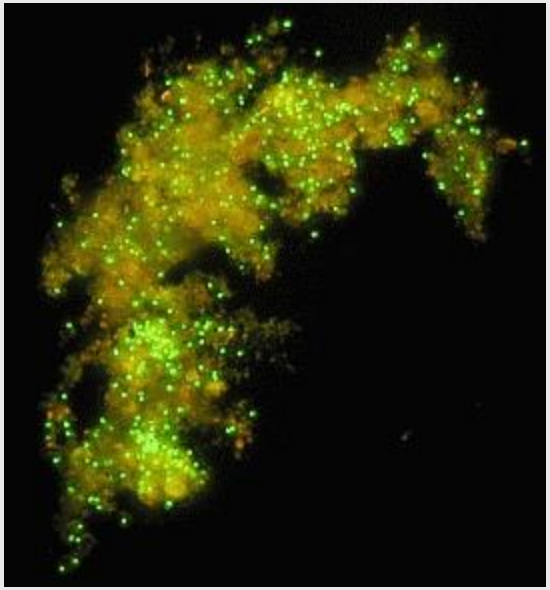
Cultivation of bacteria from the subsurface: limits and potential



Microcolonies on a sediment flake from the Black Sea

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www.icbm.de/pmbio

- **How many bacteria are "culturable"?**
- **Shallow *versus* deep biosphere**
- **How to bridge the gap between molecular and microbiological approaches**
- **Two little gifts ...**

Vibro-coring, North Sea tidal flats

How many bacteria are culturable?

- **Amann et al. 1995: 1% (>3000 citations)**

RESEARCH ARTICLE

Distributions of Microbial Activities in Deep Subseafloor Sediments

Steven D'Hondt,[1*] Bo Barker Jørgensen,[1] D. Jay Miller,[1] Anja Batzke,[2] Ruth Blake,[1] Barry A. Cragg,[1] Heribert Cypionka,[1] Gerald R. Dickens,[1] Timothy Ferdelman,[1] Kai-Uwe Hinrichs,[1] Nils G. Holm,[1] Richard Mitterer,[1] Arthur Spivack,[1] Guizhi Wang,[3] Barbara Bekins,[1] Bert Engelen,[2] Kathryn Ford,[1] Glen Gettemy,[1] Scott D. Rutherford,[4] Henrik Sass,[2] C. Gregory Skilbeck,[1] Ivano W. Aiello,[1] Gilles Guèrin,[1] Christopher H. House,[1] Fumio Inagaki,[1] Patrick Meister,[1] Thomas Naehr,[1] Sachiko Niitsuma,[1] R. John Parkes,[1] Axel Schippers,[1] David C. Smith,[1] Andreas Teske,[1] Juergen Wiegel,[1] Christian Naranjo Padilla,[1] Juana Luz Solis Acosta[1]

Diverse microbial communities and numerous energy-yielding activities occur in deeply buried sediments of the eastern Pacific Ocean. Distributions of metabolic activities often deviate from the standard model. Rates of activities, cell concentrations, and populations of cultured bacteria vary consistently from one subseafloor environment to another. Net rates of major activities principally rely on electron acceptors and electron donors from the photosynthetic surface world. At open-ocean sites, nitrate and oxygen are supplied to the deepest sedimentary communities through the underlying basaltic aquifer. In turn, these sedimentary communities may supply dissolved electron donors and nutrients to the underlying crustal biosphere.

Science 306:2216-2221 (2004)

MICROBIOLOGY

Microbial Life Breathes Deep

Edward F. DeLong

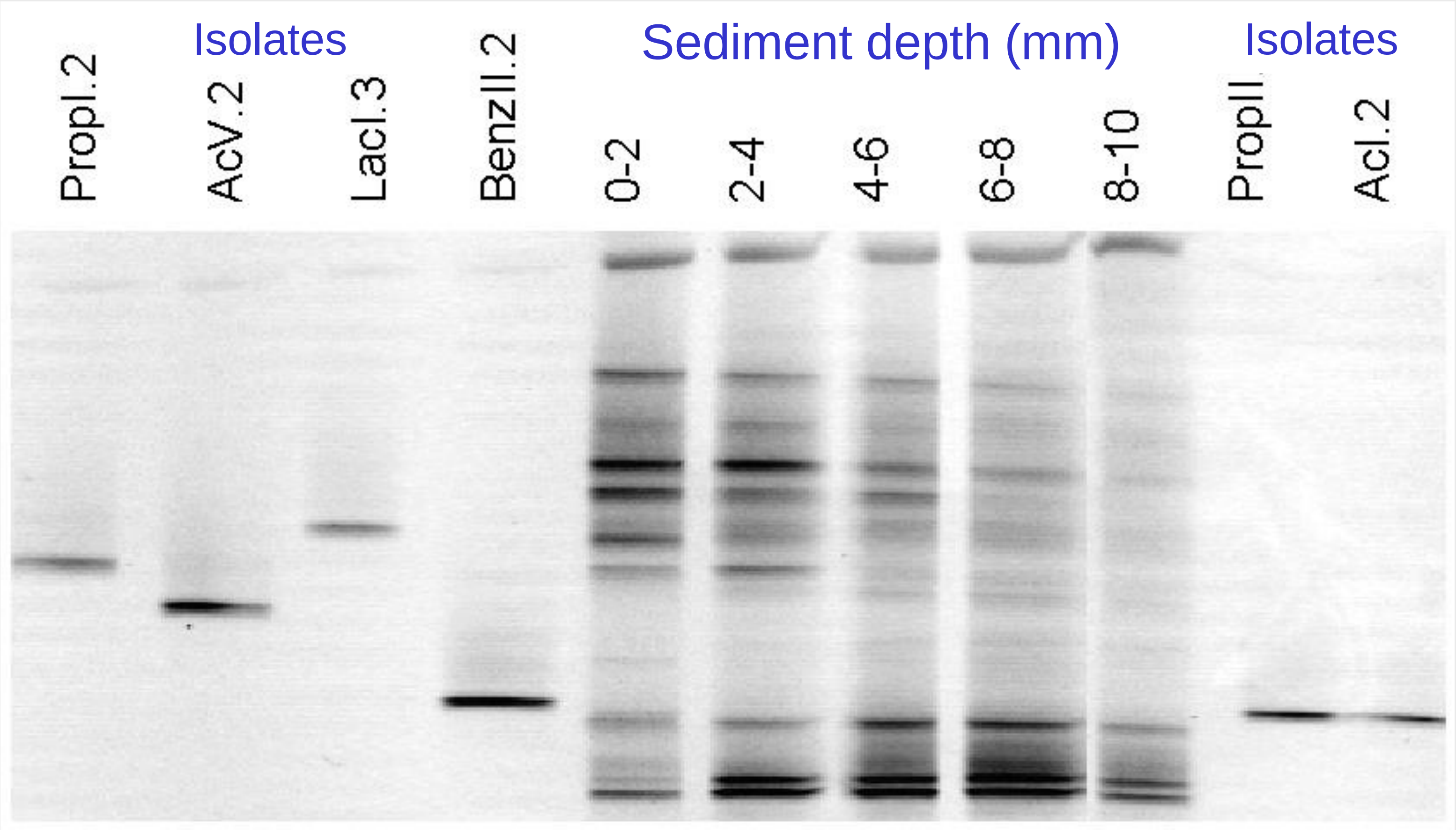
The apparent paucity of deep-sea biota led the 19th-century biologist Edward Forbes to question the very existence of life at depths greater than 550 m. Subsequent oceanographic expeditions soon laid Forbes' "azoic theory" to rest, with discoveries of a diverse and abundant marine fauna flourishing in the greatest depths of the oceans. In parallel ways, bial metabolism in subseafloor sediments.

How important are the microbial communities buried deep within the marine sediments that overlay two-thirds of Earth's surface? Counting microbes under the microscope (which does not distinguish living from dead organisms) reveals that substantial numbers of microbes must exist in deep seafloor sediments (*3*).

Exactly which microbes are responsible for the subsurface energy cycling revealed by D'Hondt *et al.* remains uncertain. Although viable sediment-associated microbes were recovered by the investigators, the relevance of these microbes to subsurface metabolism is questionable. Many of the recovered bacterial isolates form spores or are close relatives of surface-dwelling bacteria. It seems unlikely that these represent authentic deep subsurface inhabitants. Indeed, microbial survey methods that don't depend on cultivation (*5*) suggest that a quite different suite of indigenous subsurface archaea and bacteria may predominate deep within sediments (*6–8*). Such microbes may represent the indigenous, active members of deep-sea microbial communities.

- **Cultivation results questioned**

Our problem



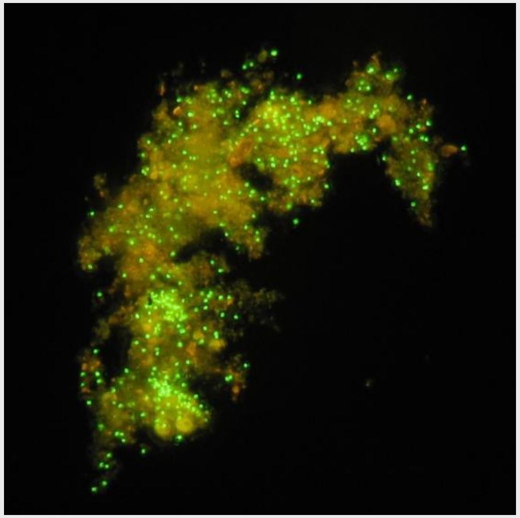
DGGE fingerprints of sulfate reducers in the uppermost centimeter of tidal-flat sediment

- **Ecological niches not provided in routine cultivation experiments**

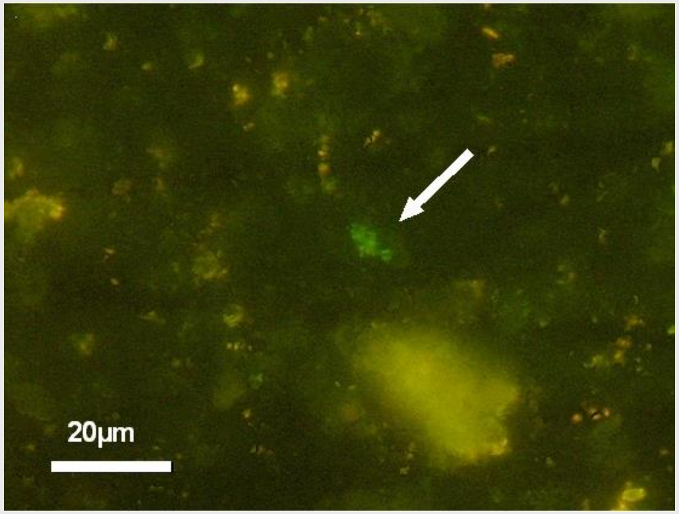
Wieringa et al. (2000) Detection of abundant sulphate-reducing bacteria in marine oxic sediment layers by a combined cultivation and molecular approach. Environ Microbiol Environ Microbiol 2:417-427

Young *versus* old sediment

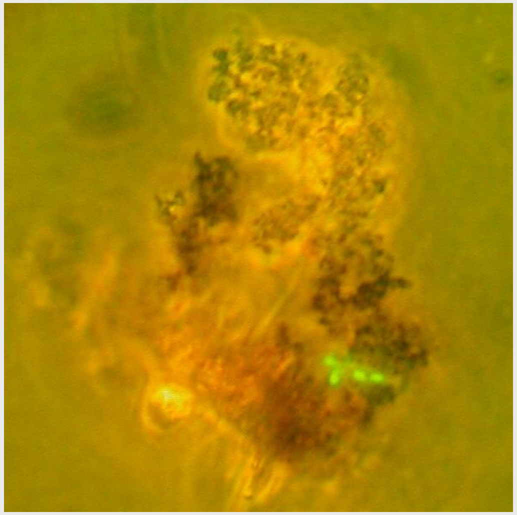
Young *versus* old sediment



Young Black Sea sapropel



Microcolony in 120 000 year old Eemian sapropel

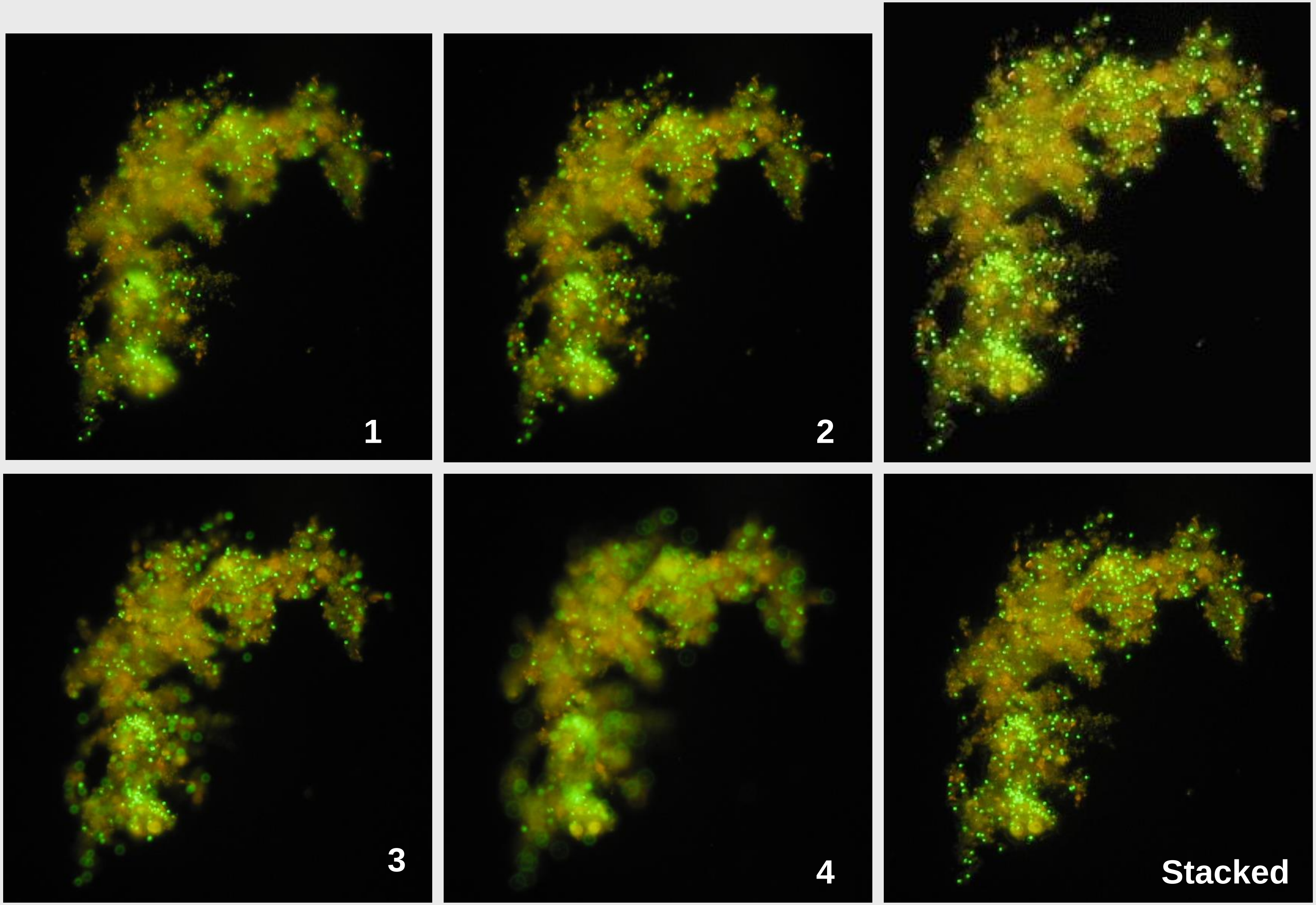


Microcolony in a culture inoculated with million-years old sediment

- **Weak signals in old sediment**

  - enhanced when cells grow

Stacking and 3-D view

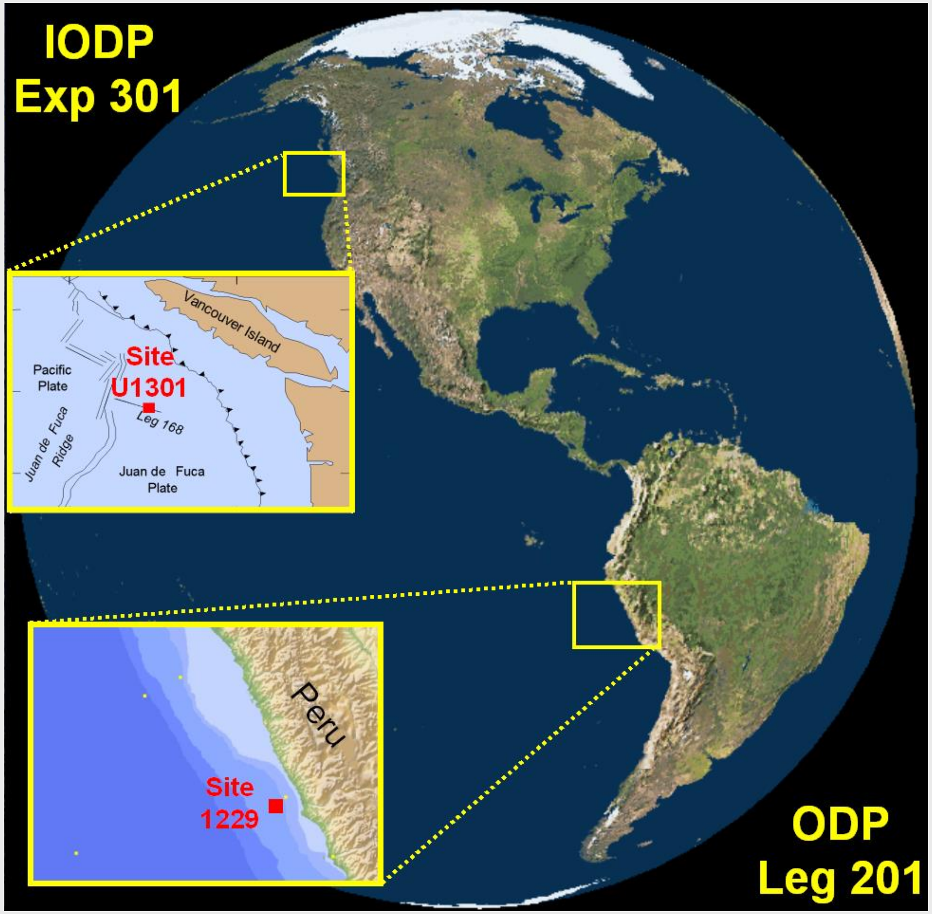


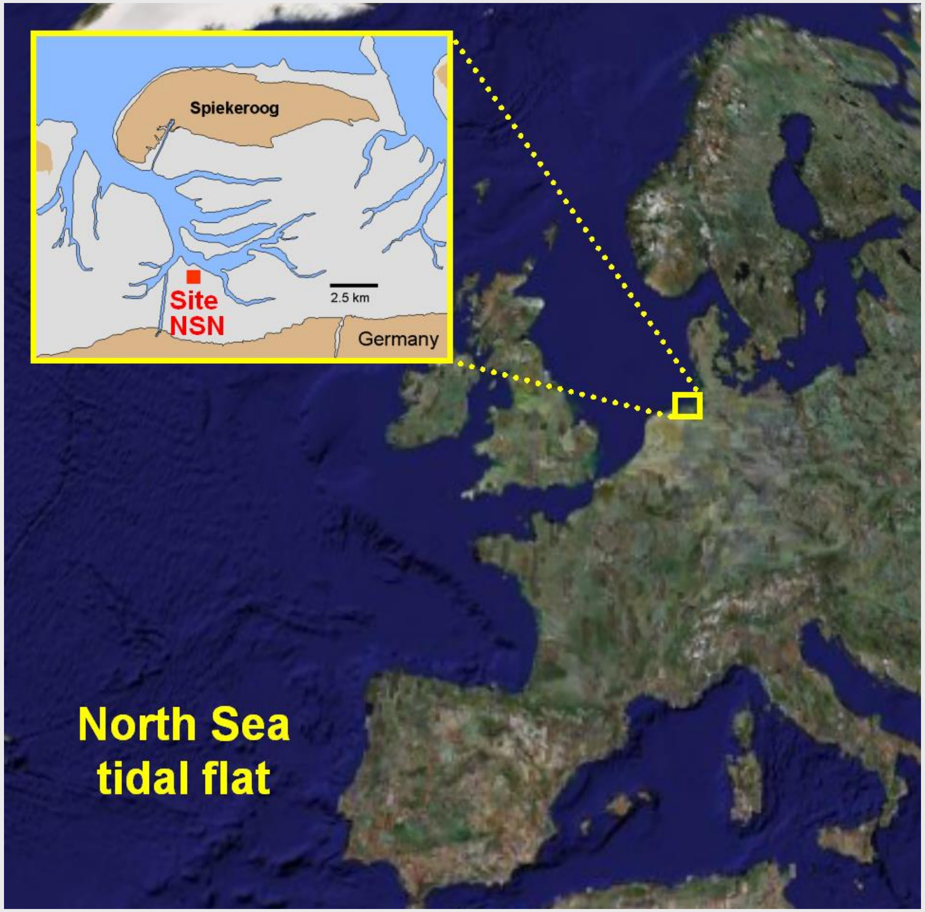
- **Freeware: www.picolay.de**

Deep *versus* shallow sediment

Deep biosphere

Shallow: Tidal flats

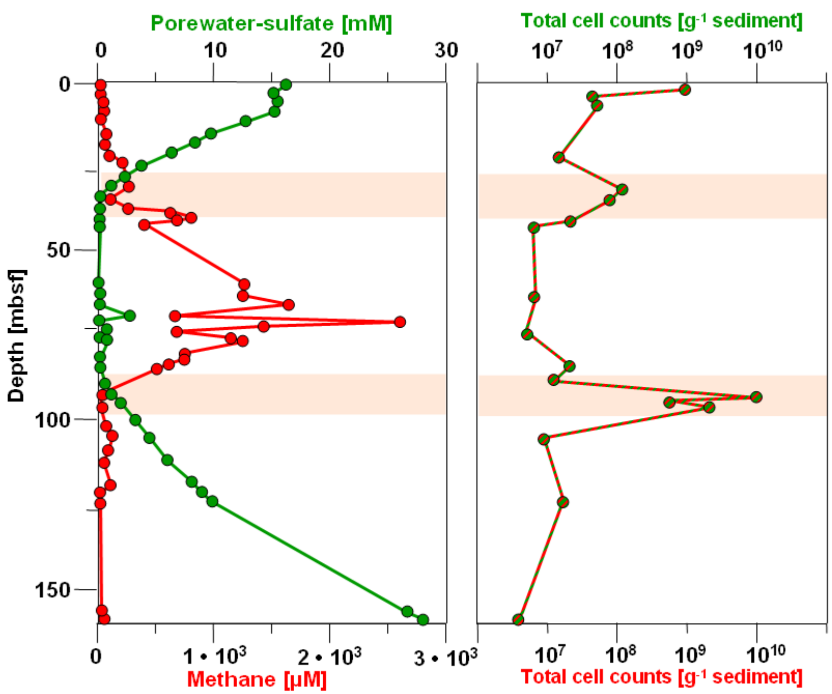




Heribert Cypionka www.icbm.de/pmbio

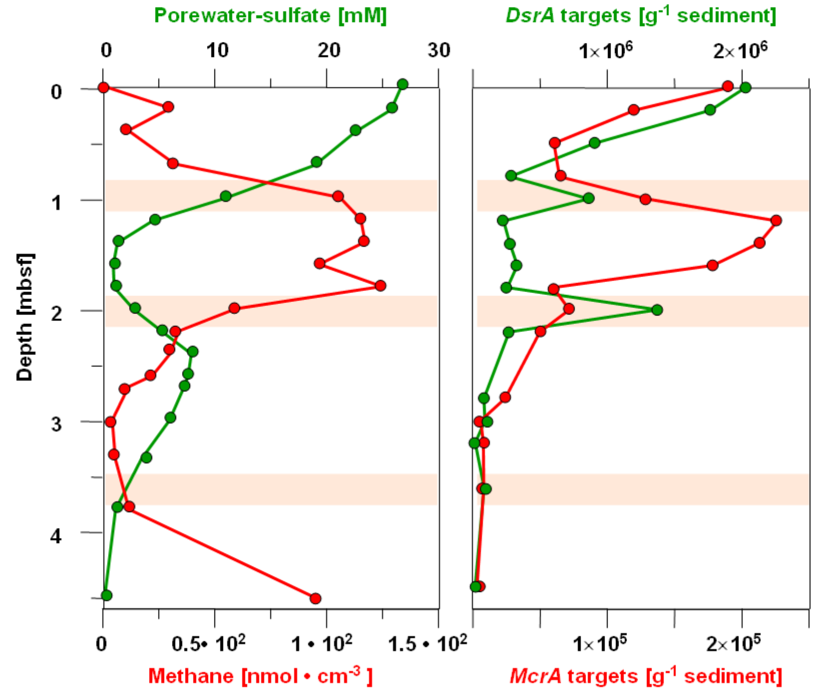
ODP Leg 201 (Site 1229)

- Continental margin, upwelling area
- Water depth: 150 m
- Temperature gradient: 14 – 20 °C
- Inflow of e⁻-acceptors by brine incursion



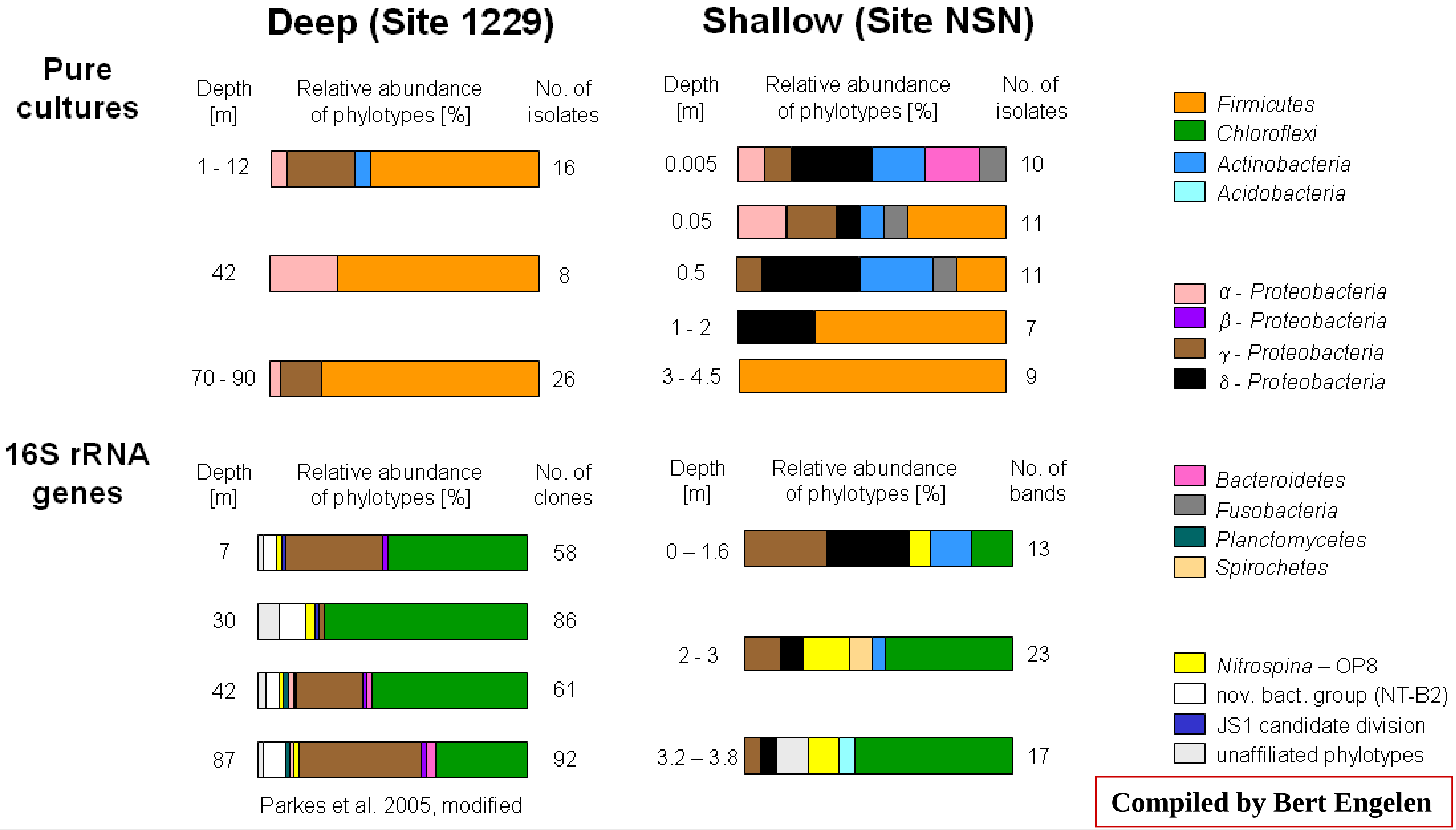
North Sea tidal flat (Site NSN)

- Coastal zone, influenced by tide
- Water depth: 0 – 2 m
- Temperature depending on season
- Lateral inflow of electron acceptors



Compiled by Bert Engelen

Wilms et al. (2007) Methane and sulfate profiles within the subsurface of a tidal flat are reflected by the distribution of sulfate-reducing bacteria and methanogenic archaea. FEMS Microbiol Ecol 59:611-621

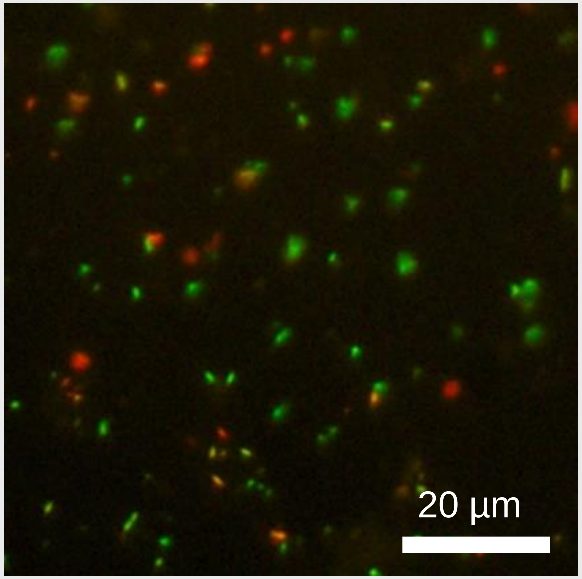


Wilms et al. (2006) Deep biosphere-related bacteria within the subsurface of tidal flat sediments. Environ Microbiol 8:709-719

Wilms et al. (2006) Specific bacterial, archaeal, and eukaryotic communities in tidal-flat sediment along a vertical profile of several meters. Appl Environ Microbiol 72:2756-2764

Unculturable, dormant or dead?

Ulrike Löffler



Soil sample: Live-dead staining with propidium iodide (red = dead) and SybrGreen (all cells)

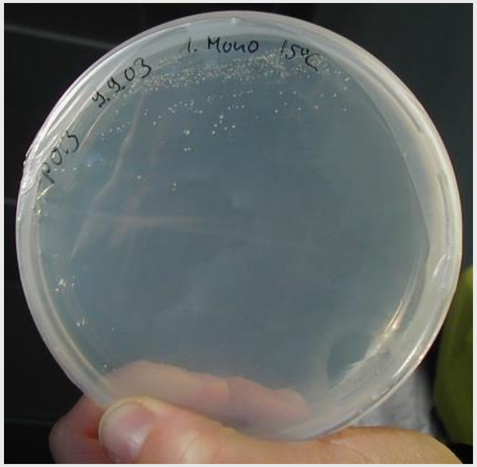
- Large portion of dead cells
- **Lower cell numbers would result in an increase of the average growth rates!**
- **Dead cells will never show up in growth experiments, spores will show up sometimes**

Conclusions (1)

- The shallow subsurface opens a window to the deep biosphere
- Many isolates and sequences point to the occurrence of typical subsurface species
- Without detailed physiological analysis we do not know specific features that discriminate terrestrial/marine and deep/shallow species
- Spores are abundant and can be analyzed with new techniques

Fichtel et al. (2007) Spore dipicolinic acid contents used for estimating the number of endospores in sediments. FEMS Microbiol Ecol 61:522–532

Cultivation approaches



Sediment isolate after 5 weeks of incubation

Cultivation success

Tidal flats or soil: > 20% of total cell counts

Mediterranean sediments: > 3 %

Peru Margin: poor

- **Cultivation is difficult.**
- **There is no 1 % limit.**

Successful cultivation approaches

- Close-to-nature conditions: Substrate concentration, pH, temperature, pressure, add particles etc.

- Use of 'non-selective' media

- Promote interactions between bacteria: add 'friends', signal molecules, avoid to separate them

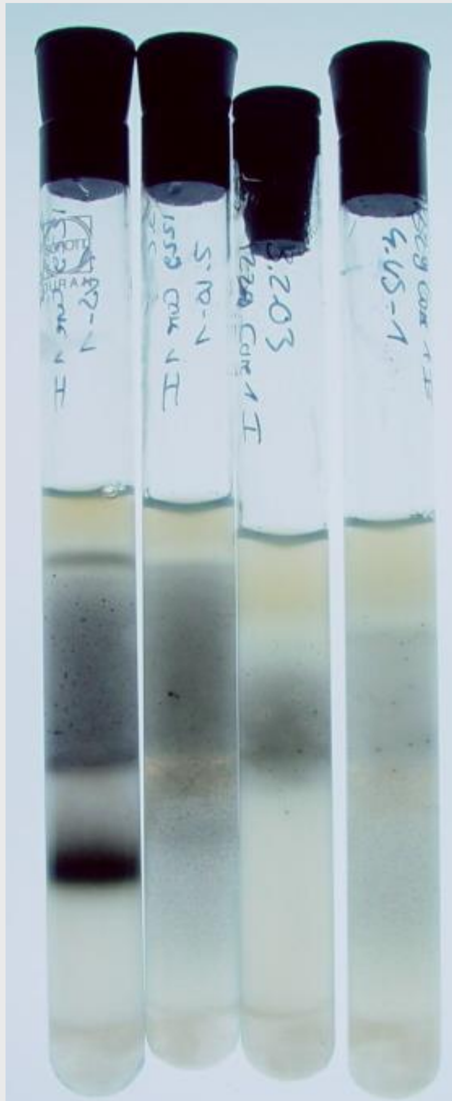
Bruns et al. (2002) Cyclic AMP and acyl homoserine lactones increase the cultivation efficiency of heterotrophic bacteria from the central Baltic Sea. Appl Environ Microbiol 68:3978-3987

- High-throughput techniques avoid competition

Bruns et al. (2003) A novel approach for high throughput cultivation assays and the isolation of planktonic bacteria. FEMS Microbiol Ecol 45:161-171

- Gradient cultures offer varying conditions

- Combine molecular and microbiological approaches!

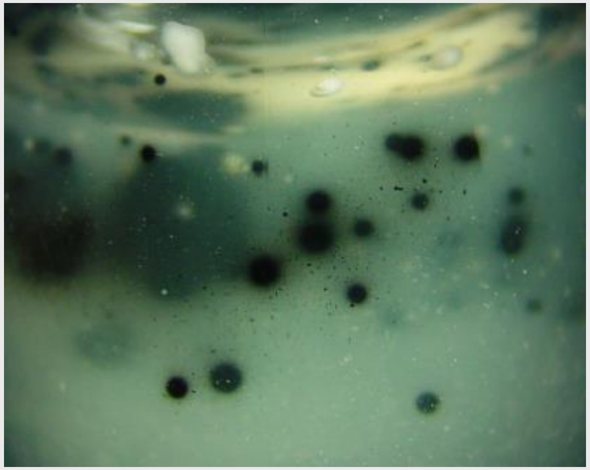


Gradient cultures

- Slowly increasing substrate concentration
- Population shift analysis

Köpke et al. (2005) Microbial diversity in coastal subsurface sediments - a cultivation approach using various electron acceptors and substrate gradients. Appl Environ Microbiol 71:7819-7830

Bacterial colonies from the deep biosphere (Peru Margin)



... pure cultures

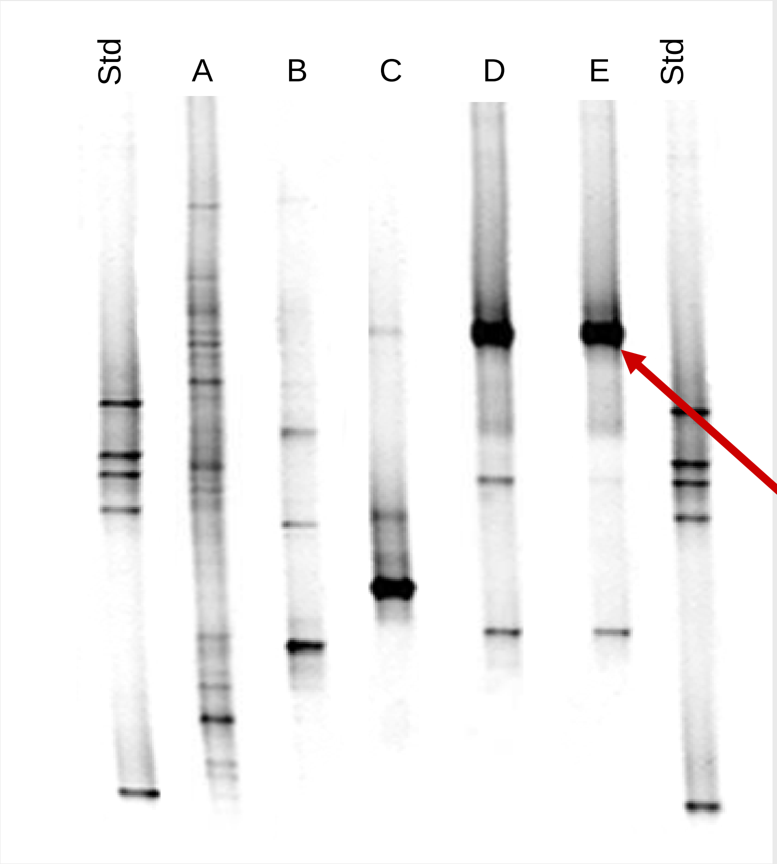
- **Many spore-formers and facultative aerobes from the deep biosphere!?**

Batzke et al. (2007) Phylogenetic and physiological diversity of cultured deep-biosphere bacteria from Equatorial Pacific Ocean and Peru Margin sediments. Geomicrobiology J 24:261-273

Molecular analysis of sediment and cultures

Mediterranean sediment with organic-rich sapropel layers

Molecular analysis of sediment and cultures



A: Untreated sapropel

B: Gradient culture with 1cm³ sapropel

C: Gradient culture with sapropel slurry

D: Liquid culture, 10 ml

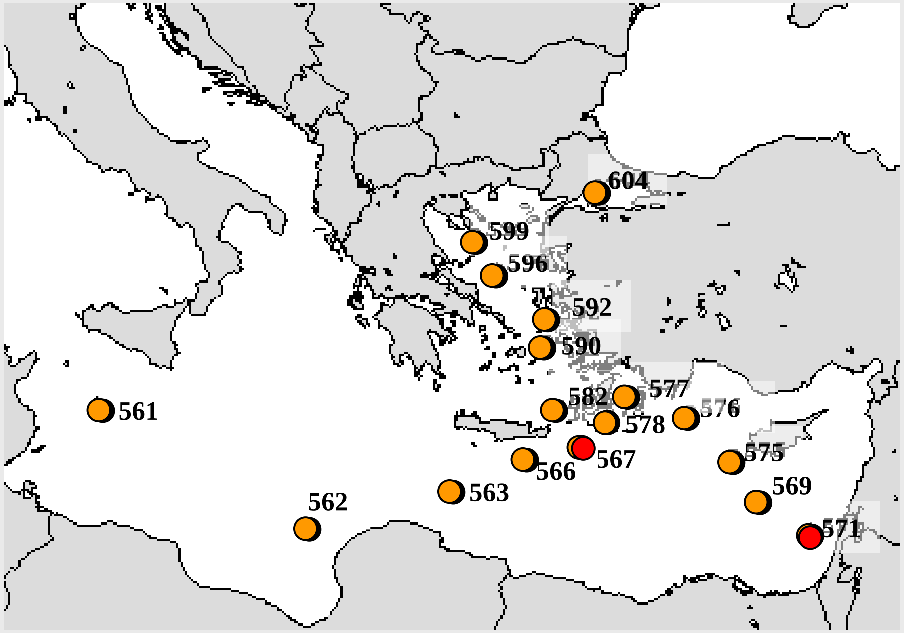
E: Liquid culture, 0.5 ml (MPN)

Rhizobium radiobacter (98% sequence similarity)

Katja Ziegelmüller

DGGE profiles of sediment (Sapropel S1) and cultures supplied with alcohols and fatty acids

- **Any variation excludes some species and gives a chance to others ...**



Rhizobium-related bacteria in old anoxic sediments

Quantitative PCR with probe

for ***Rhizobium radiobacter***

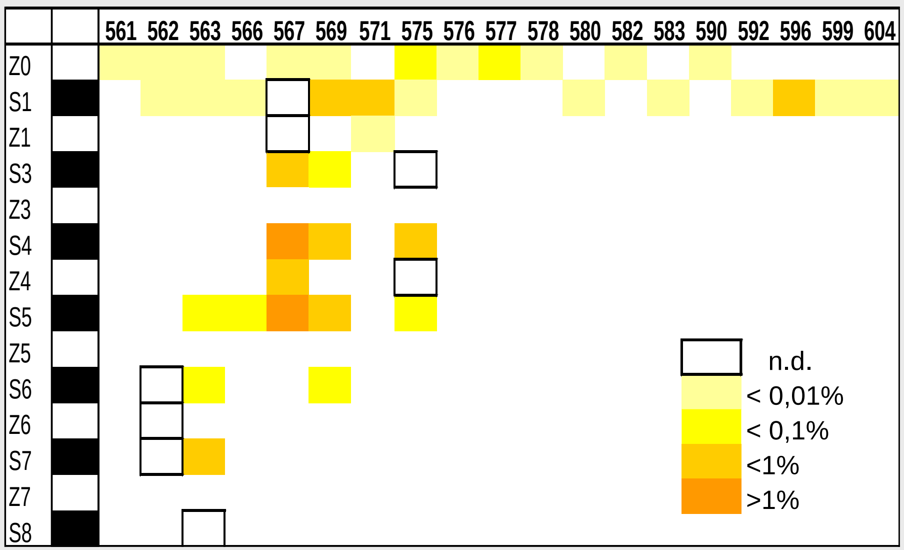
⇒ 38/46 samples positive

⇒ EUB: 10^4 - 10^7 cells/g sed.

⇒ *Rhizobium*: 10^2 - 10^5 cells/g sed.

⇒ relation: 0.001 - 5%

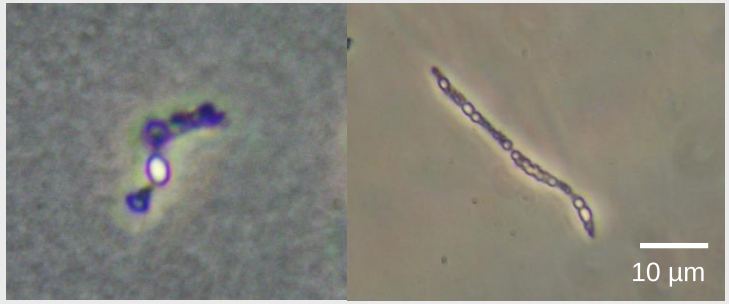
⇒ increased proportion in deep sapropel layers



Süß et al. (2006) Widespread distribution and high abundance of *Rhizobium radiobacter* within Mediterranean subsurface sediments. Environm Microbiol 8:1753-1763

Slow-growing strange morphotypes

Henrik Sass



Kerstin Herrmann

Photobacterium isolates from Mediterranean sediment

- Photobacterium spec. also found with molecular techniques in Black Sea sediment (Janin Frerichs, B. Engelen, H. Cypionka, unpubl.)

Süß et al. (2008) Two distinct *Photobacterium* populations thrive in ancient Mediterranean sapropels. Microb Ecol 55:371-383

Conclusions (2)

- Diversity is more than sequence variation, and cultivation is a hard job compared to molecular analysis:

Analysis of millions of phenotypes and niches *versus* one type of molecules (nucleic acids)

- Mother Nature cultivates them all.

- We should be able to rely on the intrinsic disposition to grow present in every organism. You`ll need ...

- ... fantasy, patience, modest expectations:

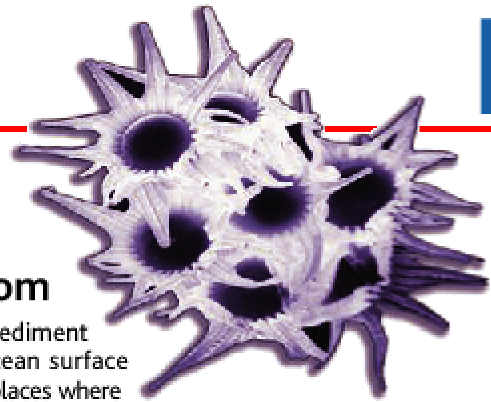
T R Y   H A R D E R

- Once you do it right, you`ll get them.

NETWATCH

IMAGES

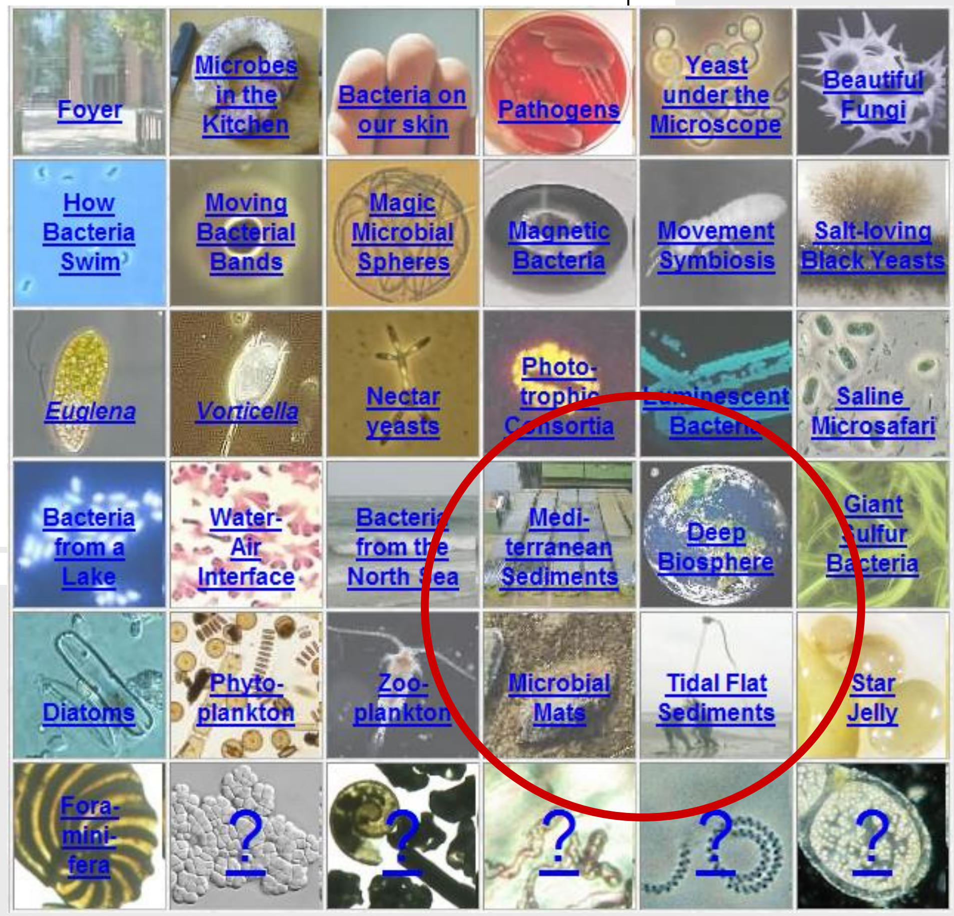
Microbes in Bloom



A briny desert lake and sediment 5000 meters below the ocean surface are just two of the unlikely places where microbes prosper. The Microbiological Garden, tended by Heribert Cypionka of the University of Oldenburg in Germany, shows off the bugs dwelling in these exotic environments and in habitats closer to home. The site features more than 20 photo essays on microbial topics. You can tag along on bug-hunting expeditions, learn how to isolate luminescent bacteria from herring, and observe the bugs that inhabit the scum on the surface of a stagnant pool. Some microbes make the gallery because of their beauty, such as these yeast spores (*Emericella stellamaris*; above) that resemble flowers.

www.microbiological-garden.net

www.sciencemag.org SCIENCE VOL 309 9 SEPTEMBER 2005



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