The deep sub-seafloor biosphere

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Discovery of the deep sub-seafloor biosphere
ODP – Ocean Drilling Program (1983-2005)
IODP – Integrated Ocean Drilling Program (2005-2013)

Intact cores with minimal contamination

Advanced piston corer
Cell numbers (AODC) in ODP sediment cores

**Conclusion:**

• Prokaryotic cells are numerous in (nearly all) deep sub-surface sediments studied

Modified from Parkes et al., Nature 1994, 371, 410-413

Parkes (2000)
Cell numbers (AODC) in ODP sediment cores

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**New record** for depth and age:
- Newfoundland margin
- 1600 m sediment depth
- 111 million years
- 60-100°C

(Roussel et al., Science, 2008)
Large populations sustained by organic matter buried over geological time

Most bacteria and archaea live under high pressure (>40 MPa)

Temperature sets the ultimate limit
Large populations sustained by organic matter buried over geological time

Most bacteria and archaea live under high pressure (>40 MPa)

Temperature sets the ultimate limit

Wilhelms et al., Nature, 2001: *Biodegradation of oil in uplifted basins prevented by deep-burial sterilization*
Global number of prokaryotic cells: $6 \times 10^{30}$

- Half of all prokaryotic microorganisms deep down in the seabed (mean sediment depth $\sim 500$ m)

- The biomass of sub-seafloor microorganisms corresponds to $1/10$ to $1/3$ of the total living biomass on Earth

Whitman et al. (PNAS, 1998)
The deep biosphere:

- what controls the cell numbers?

- does the estimate of Whitman et al. (1998) still hold?
IODP Site Survey with *RV Roger Revelle*
South Pacific Gyre, 2007
Chief scientist Steven D’Hondt

O$_2$ [µM]

oxidation of ocean crust by O$_2$

J. Fischer, F. Wenzhöfer, T. Ferdelman, S. D’Hondt, unpublished
South Pacific Gyre 2007:

- First cell counts from the most extreme low-energy region of global ocean
- Very low cell numbers (extracted cells)

Data from:
Kallmeyer et al. (2008) (black dots);
D’Hondt et al. (2003) (blue dots);
Parkes et al. (2000) (all other dots).
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Microorganisms of the deep biosphere:
Are they alive?
Who is down there?
Are they alive?

CARD-FISH:
- at least 10-30% appear to be active and alive
Schippers et al. (Nature, 2005)

Live/dead stain:
- in surface sediments: 20-80% are alive

Parkes et al. (2000)  Schippers et al. (2005)
Who is down there - bacteria or archaea?

**Q-PCR, CARD-FISH**
- mostly bacteria
  Schippers et al. (2005)

**FISH, IPL**
- mostly archaea
  Biddle et al. (2006)

Estimated cell numbers

Bacterial numbers (Log₁₀ cells cm⁻³)

Depth (mbsf)

Schippers et al. (2005)

FISH, IPL - mostly archaea
Biddle et al. (2006)

Parkes et al. (2000)

Schippers et al. (2005)
Intact polar membrane lipids - biomarkers for live cells

Top of sediment column: mostly bacteria
At depth: mostly Crenarchaeota

Lipp et al. (Nature, 2008)
Deep sub-seafloor archaea:

Marine Benthic Group B (MBG-B) = Deep-Sea Archaeal Group (DSAG)
  widespread, heterotrophs ($\delta^{13}C$)

Miscellaneous Crenarchaeotic Group (MCG)
  diverse habitats, heterotrophs ($\delta^{13}C$)

South African Goldmine Euryarchaeotal Group (SAGMEG)
  terrestrial and marine subsurface

Marine Benthic Groups A and D (MBG-A and D)
  occasional in sediments

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Marine Group I Archaea (MG-I)
  dominate in deep sea bacterial plankton
  First isolate: aerobic autotrophic ammonium oxidizer
The deep subsurface biosphere: a forest of novel, uncultured archaeal phyla emerging from the deep roots of the archaeal domain of life.

Terrestrial thermal subsurface
Terrestrial hot springs
Terrestrial Palaeosol
Freshwater Lakes
Marine water column
Marine hydrothermal vent
Marine subsurface sediment
ODP sites 1231 & 1225

Deeply-branching Archaeal lineages

Teske and Sørensen 2008. The ISME Journal 2:3-18
Bacteria or Archaea at low energy flux?

Archaea are better adapted than bacteria to extreme energy limitation (Valentine, 2007)

Low permeability membranes ⇒ lower maintenance energy
Bacteria or Archaea at low energy flux?

Archaea are better adapted than bacteria to extreme energy limitation
(Valentine, 2007)

Low permeability membranes $\Rightarrow$ lower maintenance energy
Archaeal membranes have 100-fold lower proton permeability than
bacterial membranes

**Bacteria:**
Membrane proton permeability: $10^{-2} - 10^{-1} \text{ s}^{-1}$
Membrane sodium permeability: $10^{-4} - 10^{-3} \text{ s}^{-1}$
Van de Vossenberg et al. (1995)
Search for functional genes:

Metagenomic analyses based on pyrosequencing:
(Biddle et al., PNAS, 2008)

• up to 85% of genes non-identifiable
• few sulfate reduction genes identified in sulfate reduction zone
• few methanogenesis genes identified in methanogenic zone
Organic carbon burial – fuel for the deep biosphere
Degradation rate of marine organic detritus drops by power function of time:

$$\log k = -0.95 \log t - 0.81$$

($N = 140; r = 0.987$)

J.J. Middelburg (1989)
Sulfate reduction rates, Peru shelf sediment

Experimental measurements by $^{35}$S method:

$\text{SO}_4^{2-} \rightarrow \text{H}_2\text{S}$

10 million fold drop in rate over 1 million years

J. Kallmeyer, T. Ferdelman and B.B. Jørgensen (unpublished data)
ODP Leg 201, Site 1226
4000 m water depth

Reactive-transport model:
Sulfate reduction rates are power function of depth/time

Hans Røy (unpublished)
AODC, CAR-D-FISH of bacteria
Schippers et al. (2004)

ODP Leg 201, Site 1226
4000 m water depth

Reactive-transport model:
Sulfate reduction rates are
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Hans Røy (unpublished)
- The mid-section of this deep-sea core contains $10^6$ cells cm$^{-3}$
- Sulfate reduction rate at that depth = 0.01 pmol cm$^{-3}$ d$^{-1}$
- Assuming 10% are SRM, mean cell specific sulfate reduction rate:
  - $10^{-4}$ fmol SO$_4^{2-}$ cell$^{-1}$ d$^{-1}$
• The mid-section of this deep-sea core contains 10^6 cells cm⁻³
• Sulfate reduction rate at that depth = 0.01 pmol cm⁻³ d⁻¹
• Assuming 10% are SRM, mean cell specific sulfate reduction rate:
  - 10⁻⁴ fmol SO₄²⁻ cell⁻¹ d⁻¹
  - one SO₄²⁻ ion respired per SRM every second
  - as a mean for the entire microbial cell population:
    - one electron transported per cell every second…
Bacterial respiration with sulfate:

Pure cultures: 2-10 fmol cell\(^{-1}\) d\(^{-1}\)
Growth yield = 4 g d.w. mol\(^{-1}\) acetate
Doubling time: 1 day – 1 week

Deep sub-seafloor: 10\(^{-4}\) fmol cell\(^{-1}\) d\(^{-1}\) (assuming 10% are SRM)
Doubling time: ?
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Deep sub-seafloor: 10\(^{-4}\) fmol cell\(^{-1}\) d\(^{-1}\) (assuming 10% are SRM)
Doubling time: >300-3000 years (by the same growth yield)
Natural radioactivity as alternative energy source?

The radionuclides $^{232}\text{Th}$, $^{238}\text{U}$ and $^{40}\text{K}$ form $\text{H}_2$ from radiolysis of water

Could radioactivity support life?

Blair et al. (Astrobiology, 2007)
ODP Leg 201, Site 1226
4000 m water depth

Radiolysis $\text{H}_2$ yield = 0.003 pmol cm$^{-3}$ d$^{-1}$
Visualization of
• genetic identity (hybridization)
• metabolism (uptake of $^{13}$C)

nanoSIMS (secondary ion mass spectrometer)
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Musat et al. (PNAS, 2008)
Is there genetic exchange between deep biosphere and surface:
Transport in most deep sediments is by molecular diffusion
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Molecular diffusion coefficient of inorganic ions $\sim 10^{-9} \text{ m}^2 \text{ s}^{-1}$

Diffusion time over 250 m: 1 million years

Diffusion time, $t$:

$$t = \frac{\pi L^2}{4 D_s}$$

$L = \text{mean distance}$

$D_s = \text{diffusion coeff.}$
Is there genetic exchange between deep biosphere and surface: Transport in most deep sediments is by molecular diffusion

Molecular diffusion coefficient of inorganic ions ~ 10^{-9} \text{ m}^2 \text{ s}^{-1}

Diffusion time over 250 m: 1 million years

Bacterial diffusion coefficient with swimming ~ 10^{-9} \text{ m}^2 \text{ s}^{-1}

(Fenchel, 2008)

Bacterial exchange over 250 m: 1 million years

Diffusion time, \( t \):
\[
t = \pi \frac{L^2}{4 D_s}
\]
\( L \) = mean distance
\( D_s \) = diffusion coeff.
Is there genetic exchange between deep biosphere and surface:
Transport in most deep sediments is by molecular diffusion

Molecular diffusion coefficient of inorganic ions \( \sim 10^{-9} \) m\(^2\) s\(^{-1}\)
Diffusion time over 250 m: 1 million years
Bacterial diffusion coefficient with swimming \( \sim 10^{-9} \) m\(^2\) s\(^{-1}\)
(Fenchel, 2008)
Bacterial exchange over 250 m: 1 million years
Bacterial diffusion coefficient without swimming \( \sim 10^{-12} \) m\(^2\) s\(^{-1}\)
Bacterial exchange over 10 m: 3 million years

Do they swim?

Diffusion time, \( t \):
\[
t = \frac{\pi L^2}{4 D_s}
\]
\( L \) = mean distance
\( D_s \) = diffusion coeff.
At 1 electron available per cell per second:

Motility: One flagellar motor requires 1200 protons per revolution or $10^4$-$10^5$ protons per second (Berg, 2003)

Swimming generally not possible

Metagenomic analyses show few motility genes at depth (Biddle et al., 2008)
Conclusions:

Many, perhaps most, cells in deep biosphere are alive
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Deep biosphere (i.e. most bacteria and archaea on Earth) adapted to:
• extremely low energy flux
• extremely slow growth ~ no growth
  (explains difficulties in cultivation)
Conclusions:

Many, perhaps most, cells in deep biosphere are alive.

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- extremely low energy flux
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  (explains difficulties in cultivation)

Low-energy limit for life is much lower than previously thought, ca 1000-fold lower than "maintenance metabolism"

Deep biosphere provides a new laboratory for studies of bioenergetics.
With many thanks to:

Hans Røy
Britta Gribsholt
Nils Risgaard-Petersen
Jens Kallmeyer
Tim Ferdelman
Steven D’Hondt
Andreas Teske
Fumio Inagaki
R. John Parkes
Barry Cragg
Axel Schippers
Wil Konings

- and many others

The deep biosphere strikes back: $\text{H}_2\text{S}$ alarm