Cultivation of bacteria from a six meter long core of intertidal sediment

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Introduction

Fig. 1: Some impressions of a sampling campaign in the Wadden Sea: sediment corer (A, B), measurements of chemical parameters (C)

Several investigations dealt with the microbiology of intertidal mud flats. However, only the uppermost sediment layers were investigated in detail. Little is known about the bacteria down to several meters depth, referred to as "shallow biosphere". In deeper sediment layers almost refractory organic matter is available. How do the bacteria of the "shallow biosphere" cope with this nutrient limitation? Which bacterial groups can be found along the chemical and physical gradients? How can they be cultivated? What are the most abundant species? To get first answers we analysed a 550 cm long sediment core from Gröninger Plate (East Frisian Wadden Sea, Fig. 1, 2) taken in June 2002.

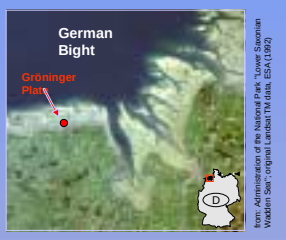

Fig. 2: Sampling site Gröninger Plate in the backbarrier tidal flat of Spiekeroog island

Materials and methods

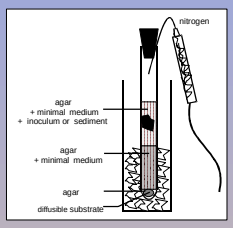

Fig. 3: Composition schema of a gradient tube

- Most propable number (MPN) counts were determined in 96 well plates using different dilute substrate mixtures and incubated under oxic or anoxic conditions.
- Cultivation in gradient tubes allowed a slow increase of substrate concentrations from the natural level and incubation occurred under anoxic conditions.
- Total DNA was extracted from original sediment, sediment embedded in gradient tubes, cultures of microtiter plates and analysed by PCR/DGGE with eubacterial primers (GC 357f, 907r).
- Total cell counts were determined with DAPI.

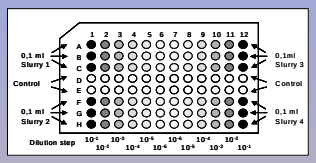

Fig. 4: Inoculation schema of MPN series in a 96 well plate

Results

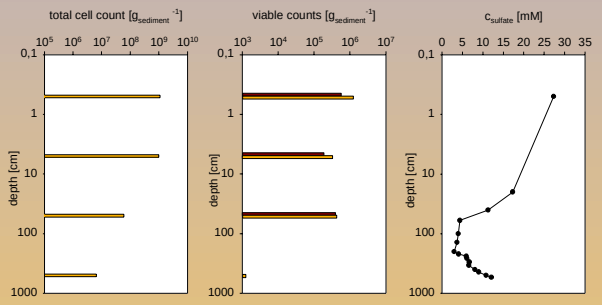

Fig. 5: Vertical profiles of total cell counts, viable counts (using momoner media and incubated under anoxic [**brown**] and oxic [**orange**] conditions) and sulfate concentration down to a depth of 500 cm.

- In the upper most 5 cm of the sediment total cell counts were high (up to $10^{-9}g_{sediment}^{-1}$), declined strongly with depth and were two magnitudes lower in 500 cm depth.
- Viable counts along the upper 50 cm were in the same magnitude and decreased about thousandfold in 500 cm depth.
- Only slight differences between oxic and anoxic viable counts were found.
- Sulfate concentrations declined rapidly within the first 350 cm and showed a clear increase below 400 cm depth.

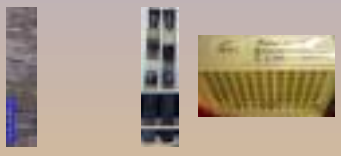

Fig. 7: Part of a sediment core (left), gradient tubes with embedded sediment (middel) and a 96 well plate with anoxic media reduced with ferrous sulfide and incubated under anoxic conditions in special incubation bags (right)

Tab. 1: Cultivation efficiency of monomer media incubated under anoxic or oxic conditions

depth [cm]	monomer-medium, anoxic	momomer-medium, oxic
0,5	0,05%	0,11%
5	0,02%	0,03%
50	0,61%	0,67%
500	0,001%	0,02%

- Using monomer media bacteria from Wadden Sea sediments can be cultivated.

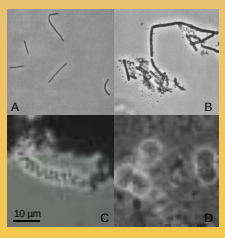

Fig. 6: Unidentified bacteria from Wadden Sea sediments cultivated in 96 well plates (A, B, C) and gradient tubes (D).

- Isolation of more than 50 pure cultures from Gröninger Plate under anoxic conditions is still in progress.
- 25 strains from Gröninger Plate were isolated under oxic conditions.

- DGGE showed clear differences between all used incubation approches.
- Sequencing of selected bands is still in progress.

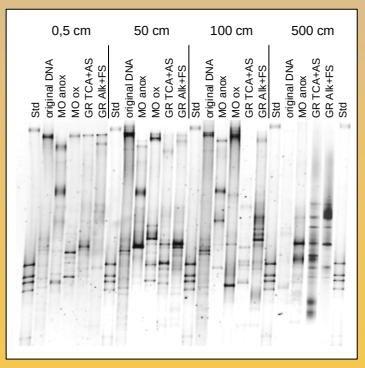

Fig. 8: Comparison of different cultivation methods using PCR/DGGE (monomer media + anoxic incubation [**MO anox**], monomer media + oxic incubation [**MO ox**] using 96 well plates; gradient tubes with different substrates: carboxylic acids + amino acids [**GR TCA+AS**] or alcohols+ fatty acids [**GR Alk+FS**]

Fig. 9: Picture (right) of the total sediment core taken on Gröninger Plate

Conclusion

- Even in 500 cm depth living microbial populations are present.
- Combination of different cultivation methods revealed a wide range of culturable microorganims. Both, using different substrate combinations and different incubation conditions are recommended for cultivation.
- The increase of sulfate concentration in deeper layers has to be correlated with microbial sulfate reduction rates to get information about microbial activities in the depth. These samples are currently under investigation.

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For detailed information about the molecular biological analysis of samples from the same core see poster number PH 013 from Reinhard Wilms *et al.*