Detecting Sulfate-Reducing Bacteria in Coastal Subsurface Sediments by Geochemical and Molecular Approaches

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Introduction

Sulfate-reducing bacteria (SRB) are the most important terminal oxidizers during the anaerobic degradation of organic matter in marine sediments. By previous cultivation experiments we were able to isolate a diverse set of SRB from tidal flat sediments down to 50 cm depth. But those from the layers beneath escaped isolation so far. However, rates of potential dissimilatory sulfate reduction indicate presence of active SRB also at greater depths.

Aim of the present study was to improve enrichment and isolation conditions for sulfate-reducing bacteria from greater sediment depth, that are likely to belong to different phylogenetic groups than the already known types. Therefore, sediment slurries were prepared and monitored over time for chemical parameters, activities and microbial community composition. These analyses shall help to understand under which conditions these SRB develop and allow to create suitable isolation set-ups.

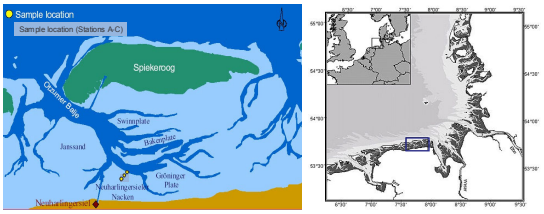

Fig. 1 Sampling location in the backbarrier area of the island Spiekeroog (Germany)

Material & Methods

Sediment cores of up to five meter length were taken on three different sites along a 100 meter transect. Station A was situated closest to the adjacent tidal creek. Station C was the most remote one (Fig. 1). Pore water concentrations of sulfate and acetate were determined by ion chromatography. Sulfate reduction rates were measured by cold chromium distillation (Kallmeyer et al. 2004). Total cell counts were obtained by staining with SybrGreen I. Most probable numbers (MPN) were determined using different substrate mixtures or acetate as sole carbon source to promote growth of different types of sulfate-reducing bacteria.

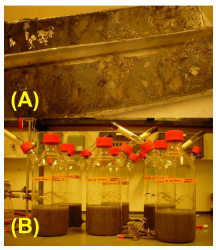

Fig. 2 (A) Part of the sediment core, (B) Anaerobic sediment slurries

Sediment slurries were prepared in duplicates with artificial seawater and amended with substrate mixtures of short chain fatty acids (SCFA), intermediates of tricarboxylic acids (TCA compounds) or remained unamended as a control (Fig. 2). The inoculum was taken on **Station B** from a depth of approx. 2 to 2.5 meters below surface. The consumption of sulfate and substrates was recorded with time and correlated to the concomitantly analyzed microbial communities.

Results

- Small-scale variability in lithology of adjacent sampling sites was reflected by pore water profiles, e.g. of sulfate (Fig. 3)
- At station A and B sulfate peaks were found in a porous layer mainly consisting of mussel shells, underlain by compacted mud of low hydraulic conductivity. This peak did not show up at station C where the deep layers were virtually mud-free.
- Sulfate reduction rates measured on Station B and viable cell counts (Fig. 4) indicated the presence of active sulfate reducing bacteria in sediment layers beneath 50 cm
- DGGE analysis of enrichment cultures showed successional changes in bacterial community composition as well as differences correlated to the respective substrate combinations offered (Fig. 5)

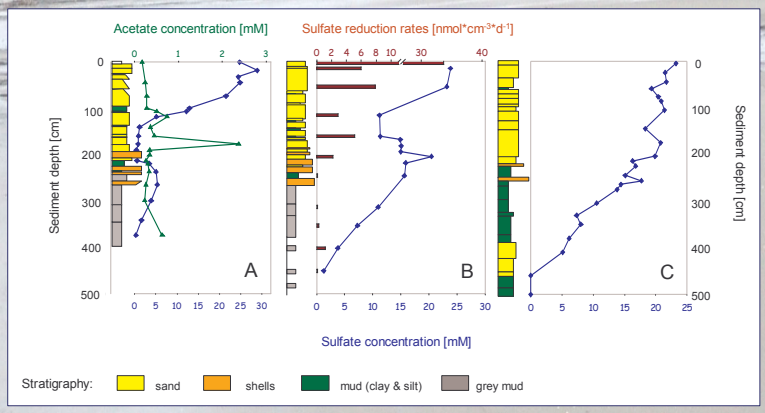

Fig. 3 Simplified stratigraphy and depth profiles of sulfate, acetate and sulfate reduction rates (SRR) for three locations on a transect of approx. 100 meter (A – C)
(Station B, C: no detectable acetate; Station C: potential sulfate reduction below detection limit, in preparation for Station A)

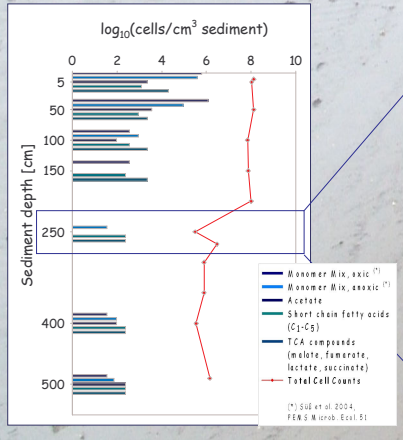

Fig. 4: Total cell counts and viable counts obtained with different substrate mixtures in microtiter plates (after 2 months of incubation)

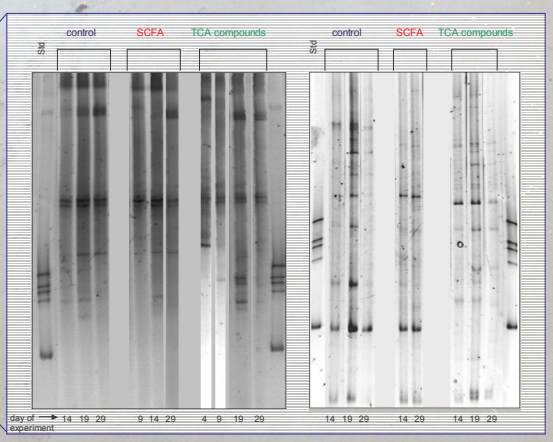

Fig. 5 DGGE patterns of sediment slurries amplified with specific primers for *Bacteria* (left) and dissimilatory sulfite reductase A -gene (*dsrA*) (right)

Conclusion

- Presence of active sulfate-reducing bacteria in subsurface sediments of a tidal flat system was substantiated by their **successful enrichment** and molecular detection
- Time- and substrate-dependent differences in DGGE patterns will be analysed in detail by identifying enriched organisms and their selective cultivation conditions

This project was funded by the „Deutsche Forschungsgemeinschaft“ (DFG). The authors would like to thank B. Köpke for help during analysis and fruitful discussions.