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Introduction

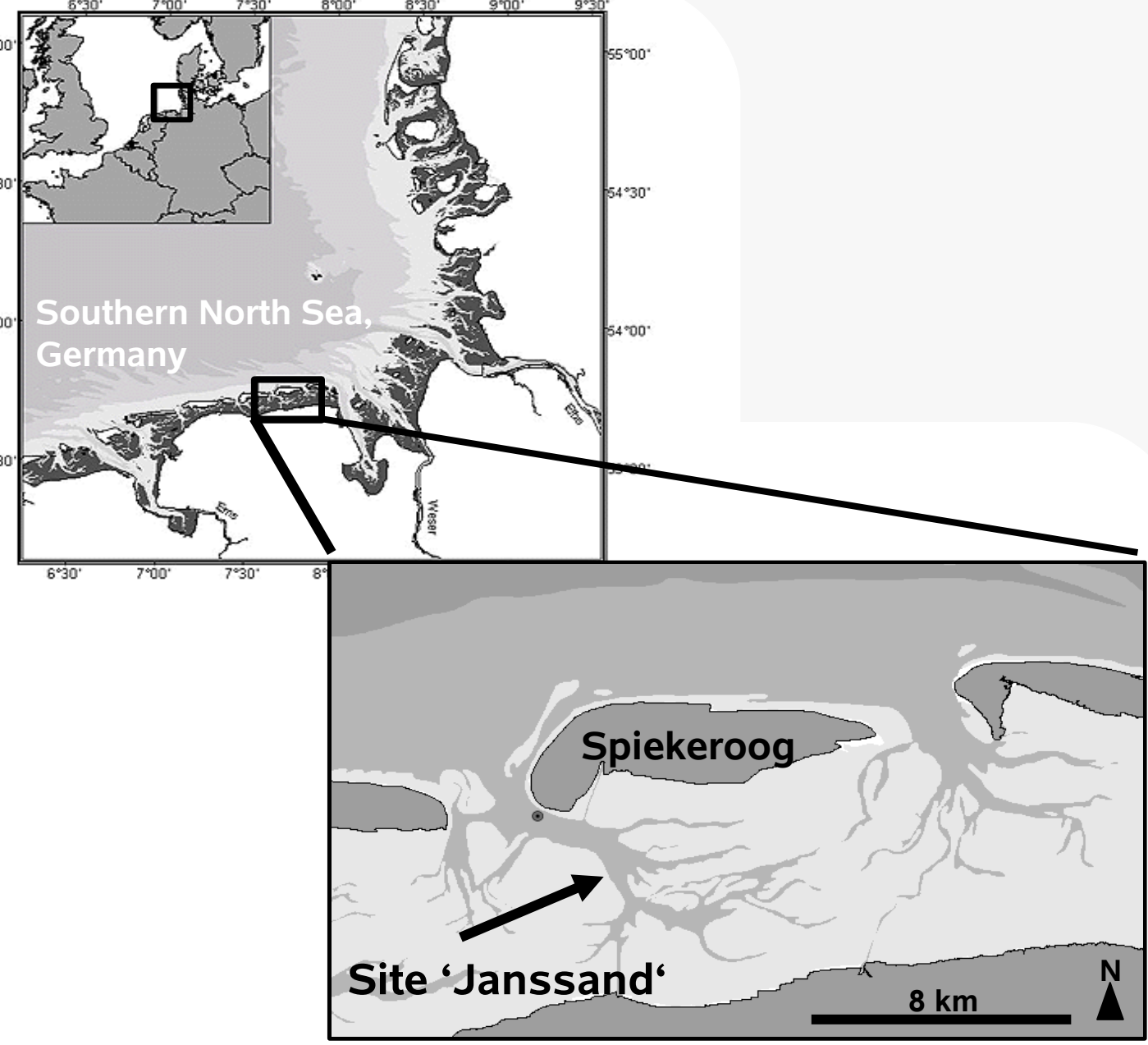
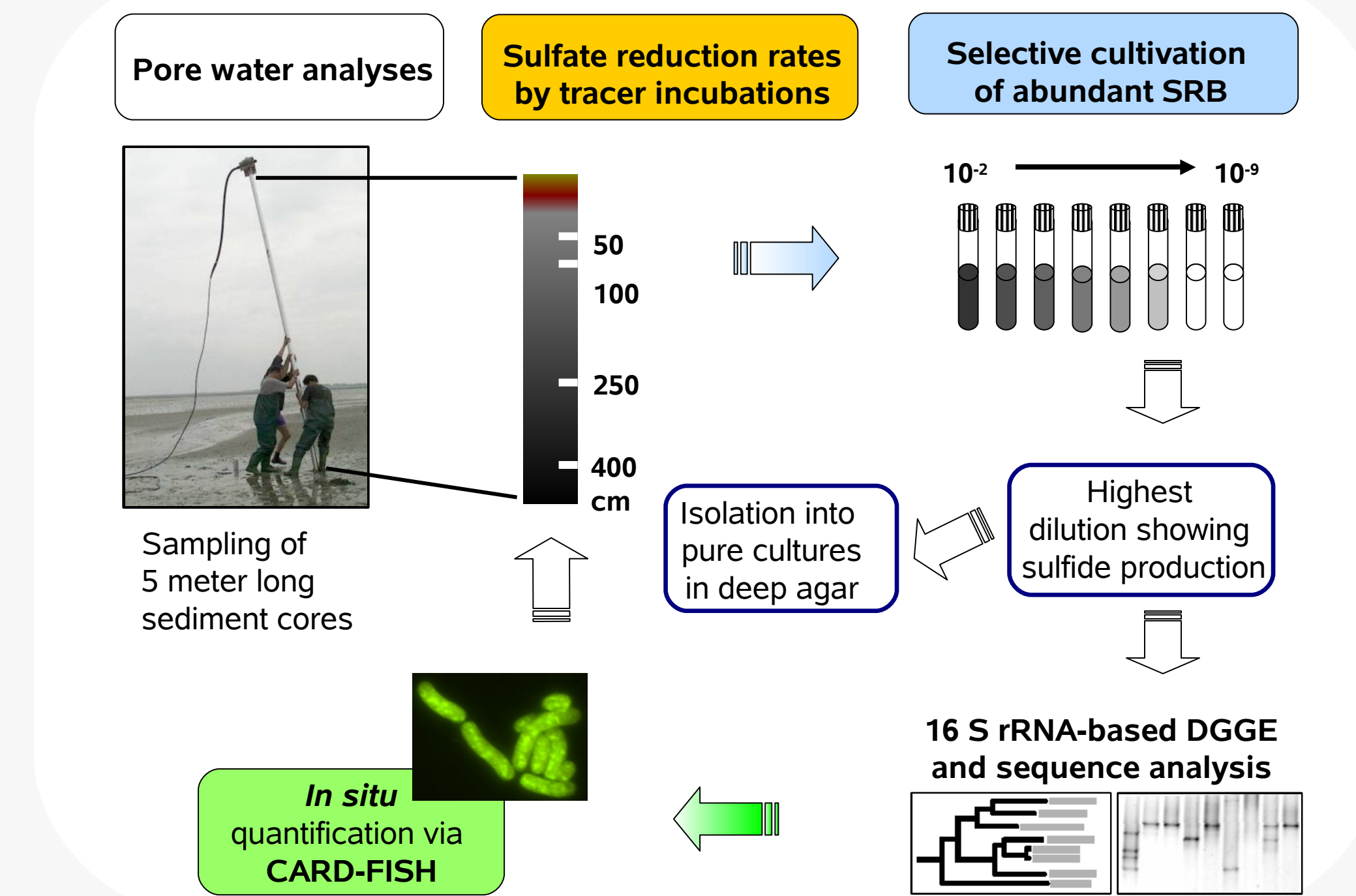


Fig. 1 Sampling site in the backbarrier of the island Spiekeroog, Southern North Sea, Germany

Recent studies on intertidal flats give first insights into the structure and distribution of microbial communities in sediments up to several meters depth (Köpke et al. 2005, Wilms et al. 2006).

Sulfate-reducing bacteria (SRB) were detected by both cultivation and molecular methods. But still little is known about their activity and abundance in these deep marine sediments. Additionally, only few SRB from these depths were brought into pure culture so far.

Here, we present geochemical and activity analyses combined with the selective cultivation of abundant SRB and their *in situ* quantification.



Results

Microbial activity & Pore water chemistry

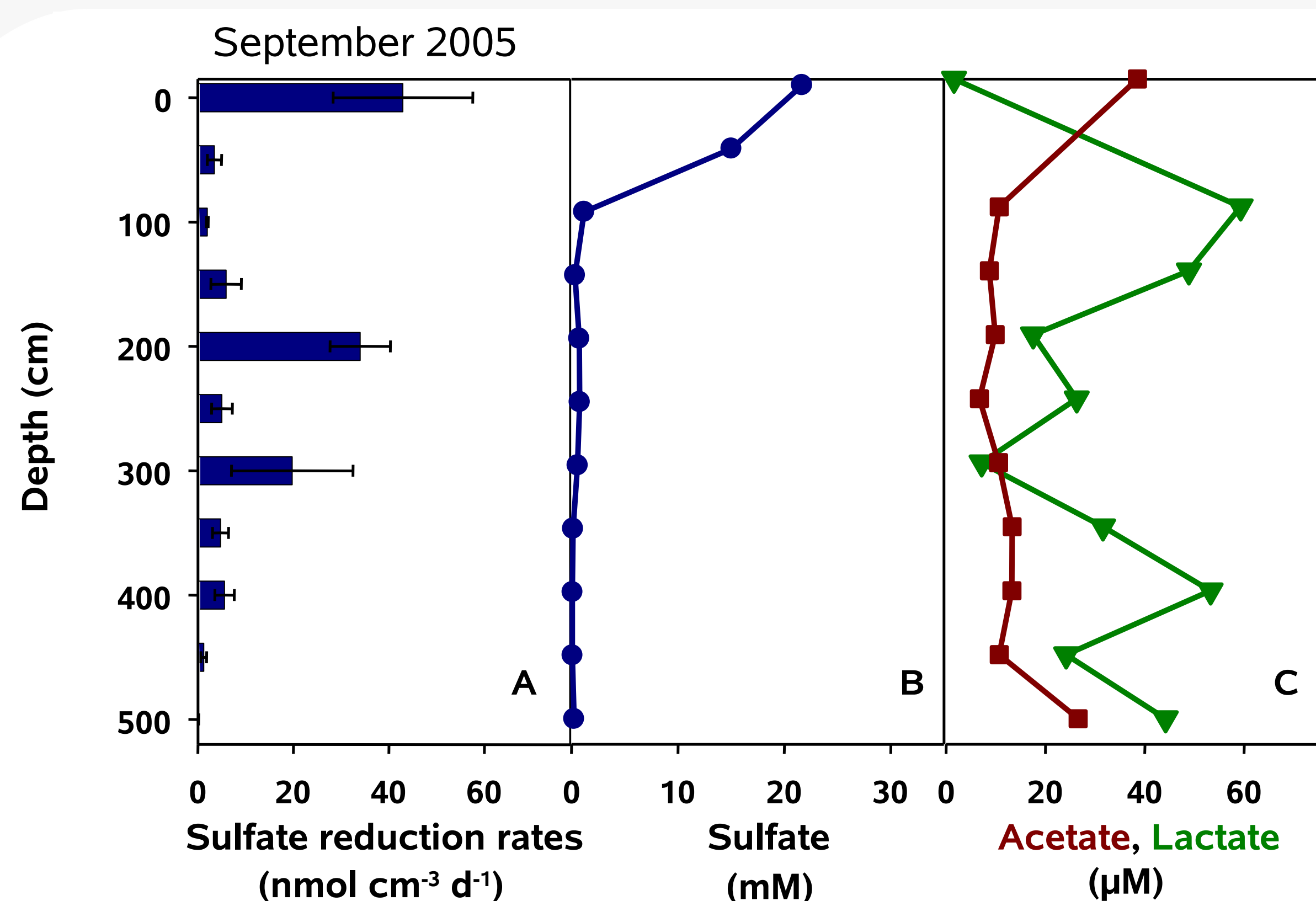


Fig. 2 (A) Microbial sulfate reduction rates; (B) Pore water concentrations of sulfate; (C) Pore water concentrations of acetate and lactate as potential substrates for microbial sulfate reduction

- Activity of SRB was highest in surface sediments, but was still detectable in several meters depth (SRR, Fig. 2A)
- In some layers, rates of microbial sulfate reduction were close to those measured in the top-layer

- Concentrations of potential substrates for microbial sulfate reduction were generally low (acetate: < 15 μM) or, for lactate, showed local minima at several depths (Fig. 2C)

Cultivation of abundant SRB

- Significant sulfide production and, therefore, growth of SRB was observed in cultures from all depths and with lactate, acetate, or hydrogen as electron donor up to dilutions of 1:10⁶

- Sequencing and phylogenetic analysis of DGGE bands received from the highest sulfide-positive dilutions revealed 13 partial 16S rRNA genes, most of them affiliating with marine SRB of the *Deltaproteobacteria* (Fig. 4)

In situ abundance of cultured SRB

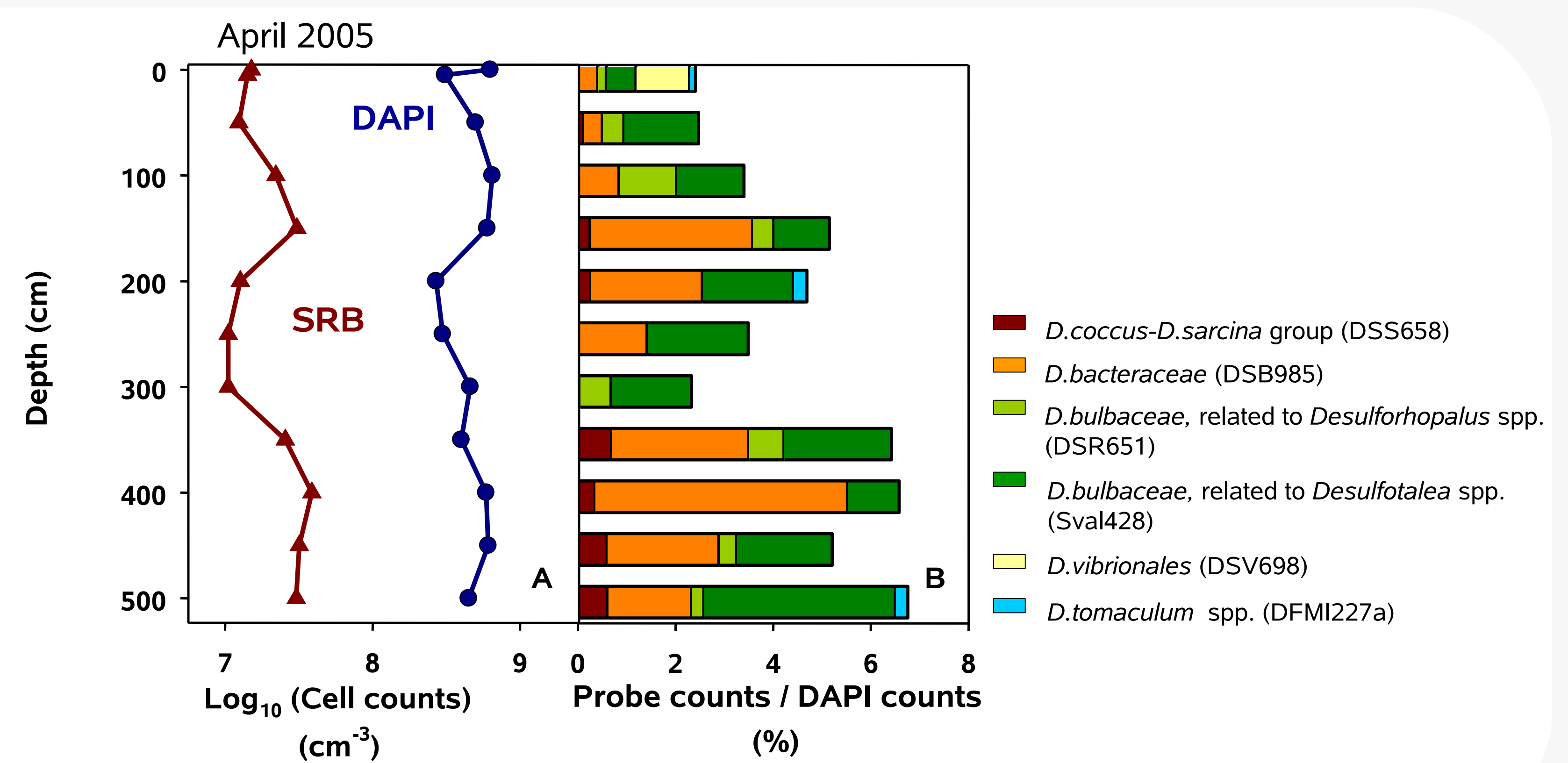


Fig. 3 (A) Total DAPI Counts and absolute numbers of SRB as detected with CARD-FISH; (B) Relative abundance of different phylogenetic groups of SRB

- Both total cell counts and SRB counts varied only slightly with respect to the sediment depth (Fig. 3A)
- SRB were found in all sediment layers accounting for up to 7 % of total cells

- The most abundant SRB were members of the *Desulfobacteraceae* and the *Desulfobulbaceae* that accounted for at least two-thirds of all detected SRB in deep sediment layers (Fig. 3B)

Isolation of SRB into pure cultures

- So far, 10 SRB originating from different sediment depths were isolated into pure cultures with lactate, acetate, or hydrogen as electron donor
- Their phylogeny and physiology is presently under investigation

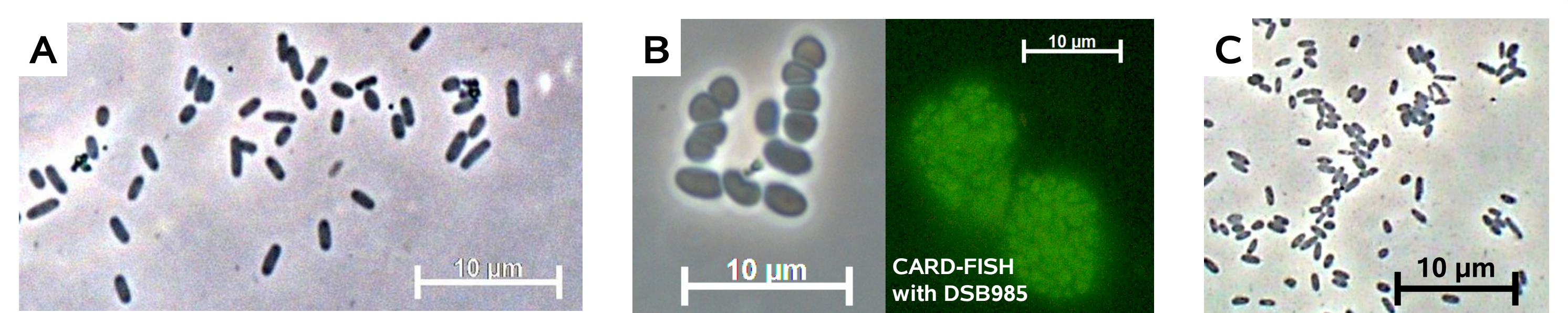


Fig. 5 Micrographs of representative morphotypes from pure cultures (A) 100 cm, hydrogen; (B) 250 cm, acetate, (C) 400 cm, hydrogen

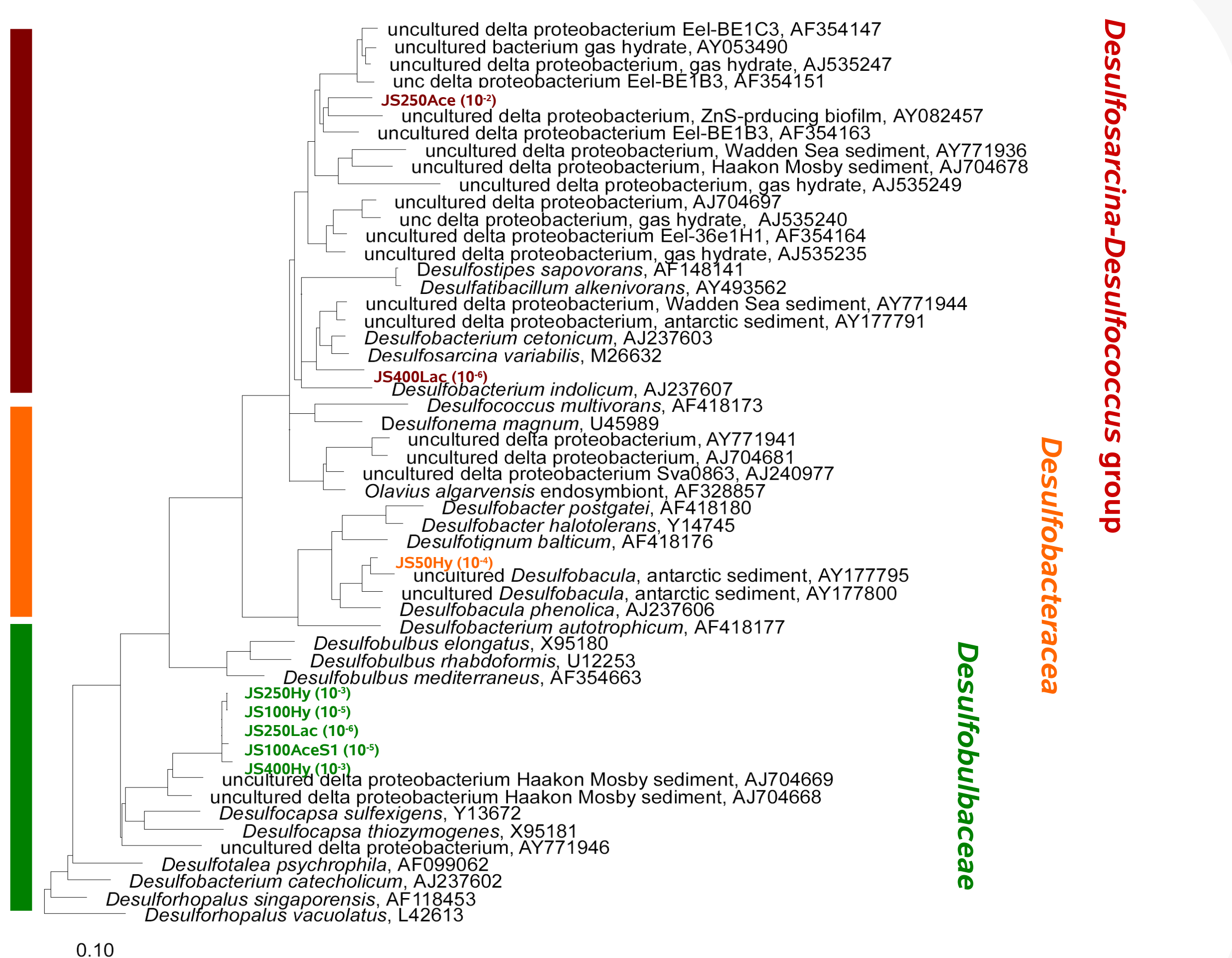


Fig. 4 Phylogenetic affiliation of extracted DGGE bands (~500bp) received from the highest dilutions showing sulfide production. Sequences from this study are highlighted. The scale bar corresponds to 10% estimated sequence divergence.

Conclusions

- Activity of sulfate-reducing bacteria in deep Wadden Sea sediments was demonstrated via radiotracer measurements
- It was shown that active sulfate-reducing bacteria form a major fraction of the microbial community particularly in deep sediment layers
- We successfully cultivated sulfate-reducing bacteria affiliating with as yet uncultured organisms exclusively detected by molecular approaches
- Frequent cultivation of members of the *Desulfobacteraceae* and *Desulfobulbaceae* in dilution cultures was mirrored by their abundance *in situ*