Revealing the importance of endospores in marine sediments

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Dipicolinic acid – a biomarker for endospores

Endospores are bacterial resting stages being able to remain viable for long periods of time. Consequently, they can be expected to accumulate in sediments during burial and may contribute significantly to total cell counts. The number of spores in sediments has only rarely been quantified because of methodological problems and consequently little is known about the quantitative contribution of endospores to the total number of prokaryotic cells. This emphasizes the need for a new cultivation-independent approach for the quantification of bacterial endospores in sediments.

In the present study, dipicolinic acid (DPA), a biomarker for endospores, was used to quantify endospores in marine sediments. Sediment cores of up to 6 m length were collected from different sites in the backbarrier tidal flat of the island of Spiekeroog in the southern North Sea and analyzed for their DPA content to determine endospore depth profiles.

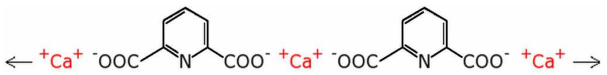
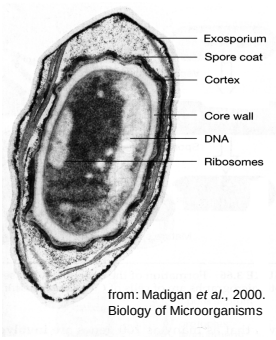


Fig. 1. Ca^{2+} complex of dipicolinic acid (DPA, pyridine-2,6-dicarboxylic acid), making up 6-18% of dry weight of bacterial endospores [1].



from: Madigan *et al.*, 2000. Biology of Microorganisms

Fig. 2. Transmission electron micrograph of a mature endospore of *Bacillus megaterium*. DPA is located in the spore protoplast (core) which is surrounded by the very resistant spore coat layers.

Quantification of DPA in sediments and conversion into endospore numbers

For quantification of DPA a high-performance liquid chromatographic method with indirect fluorescence detection has been developed [2]. After separation on a reversed-phase column, a post-column reagent with terbium chloride was added for complexation of DPA. Terbium monodipicolinate complexes formed were quantified by their characteristic fluorescence maxima. Parameters of post-column complexation were optimized to achieve a detection limit of 0.5 nmol DPA l^{-1}, corresponding to about 10^3 endospores per ml.

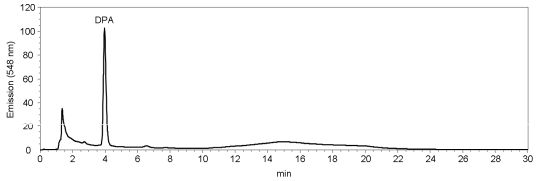


Fig. 3. Fluorescence chromatogram of a sediment extract: DPA was completely separated from the fluorescent sediment matrix and could be quantified without further sample preparation.

For conversion of sediment DPA contents into endospore numbers an average DPA content of $2.24 \cdot 10^{-16}$ mol per spore (SD: $0.63 \cdot 10^{-16}$ mol) was assumed. This factor was determined from spore DPA contents of six strains isolated from the investigated tidal flat area [3].

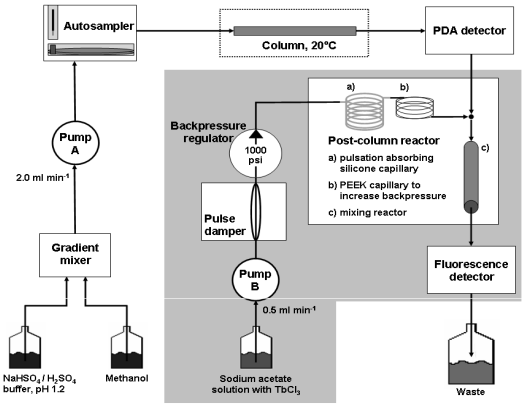


Fig. 4 HPLC system for post-column complexation and fluorimetric determination of DPA.

High variations in endospore numbers within tidal flat sediments

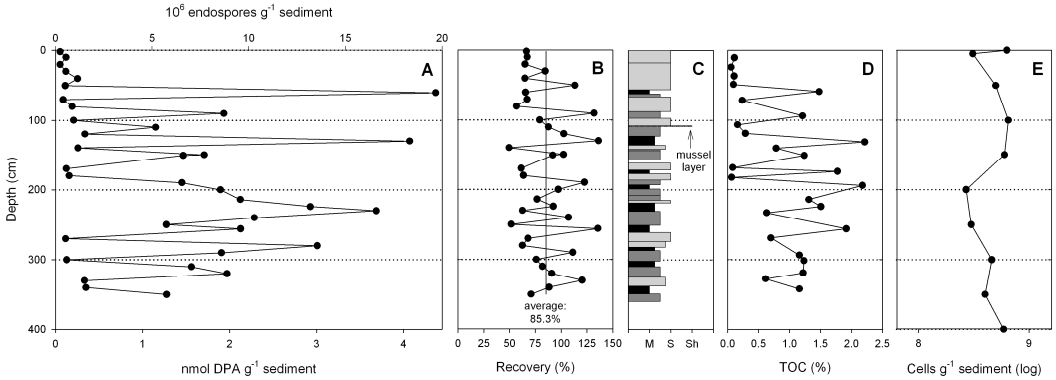


Fig. 5. Depth profiles of microbiological and sedimentological parameters in a core taken from the backbarrier tidal flat.

A) Endospore numbers estimated from sediment DPA contents.

B) Recovery determined by spiking of sediment aliquots with DPA.

C) Lithological profile of the core. The width of the bars represents the grain size (M: mud, S: sand, Sh: shells). Black mud (black), mud/sand mixed sediments (dark gray) and sand (light gray).

D) Total organic carbon (TOC) content.

E) Total cell counts [4]

DPA contents of the sediment samples ranged from 0.02 to 4.4 nmol DPA g^{-1} sediment corresponding to $1 \cdot 10^5$ to $2 \cdot 10^7$ spores g^{-1}. Distribution of endospores did not reflect a continuous depth profile, but seemed to be strongly influenced by lithology. Highest endospore numbers were determined in thin black mud layers. Significantly lower spore numbers were found in sandy sediments. The contribution of endospores to the sedimentary microbial community was estimated not to exceed 1% of total cell counts in the upper 50 cm of the sediment column at all sites. However, in a few meters depth endospores were estimated to account for up to 10% of total cell counts [5].

Conclusions

Quantification of DPA permitted a cultivation-independent determination of endospore numbers in marine sediments:

- **More than 10^7 spores g^{-1} sediment were determined in thin black mud layers**
- **Endospores represented up to 10% of total cell counts in a few meters depth**

➡ **Do endospores contribute substantially to the deep biosphere ?**

References

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