

# THE MASSIVE CONTRIBUTION OF BACTERIAL ENDOSPORES TO THE MARINE DEEP BIOSPHERE

L. Wörmer<sup>1</sup>, T. Hoshino<sup>2</sup>, B. Viehweger<sup>1</sup>, Y. Morono<sup>2</sup>, F. Inagaki<sup>2</sup>, K.-U. Hinrichs<sup>1</sup>

<sup>1</sup>MARUM, University of Bremen, Bremen, Germany. <sup>2</sup>Kochi Institute for Core Sample Research, JAMSTEC, Kochi, Japan

## Introduction

Scientific drilling during the last decades has evidenced the abundance of Earth's deep microbial biosphere in terrestrial and marine realms. However, it remains to be explained how these communities deal with progressive burial and the associated gradual energy starvation, i.e. how they achieve long-term survival on geological time scales.

Bacterial endospores are specialized, metabolically inactive, dormant cells that are structurally differentiated from vegetative cells and present specific resistance and persistence strategies. Sporulation is triggered by a variety of stimuli, the main one being prolonged starvation. At the same time, endospores have the ability to monitor the habitat to resume active growth when the conditions become favourable. Endospore formation is exclusive to the phylum *Firmicutes* and widespread among its different physiological groups. As *Firmicutes* are considered important members of the subseafloor biosphere, endospores may constitute a critical long-term survival strategy in this ecosystem.

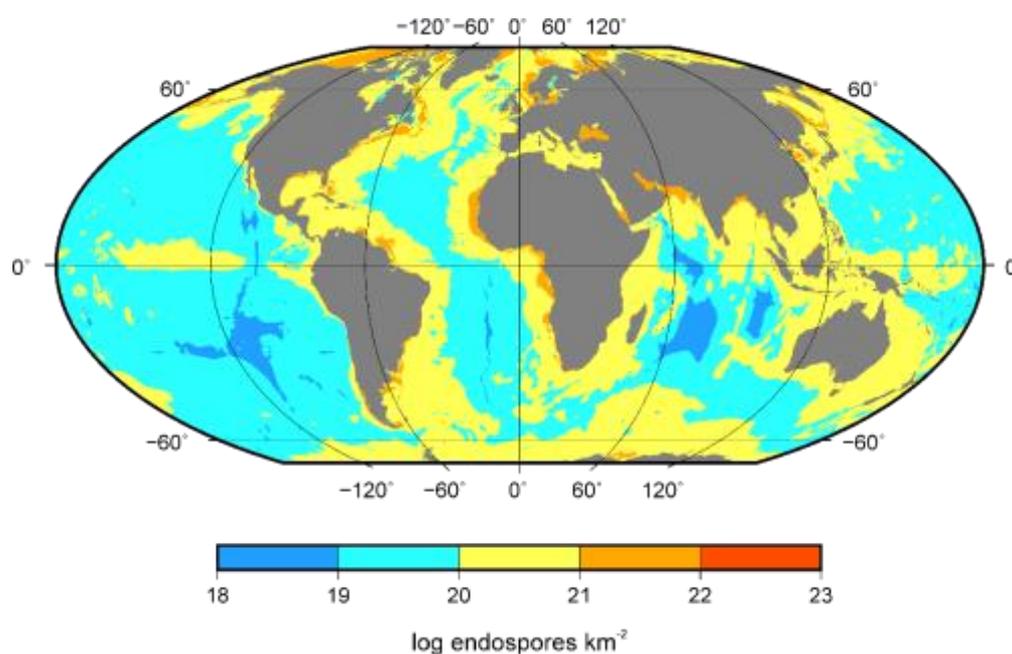
Due to a lack of reliable analytical methods, endospores have been poorly accounted for in previous surveys of the subseafloor microbiome. However, with the implementation of HPLC-based protocols for the detection of the diagnostic biomarker dipicolinic acid (DPA) (Fichtel et al., 2007), accurate and reliable quantification of intact bacterial endospores has become available. In the present study, we employed this approach to unravel the global abundance of these resistance forms in the marine deep biosphere.

## Results

More than 300 sediment samples spanning a wide range of depths and depositional settings and representing a good geographical coverage were analyzed for bacterial endospore abundance (DPA-based), vegetative cell numbers and total organic carbon content.

Following the typical trend observed in marine sediments, the number of vegetative cells in our study sites decreased with depth. Endospore abundance on the other hand remained relatively constant in a range between roughly  $10^5$  and  $10^6$  units per g. Only some surface samples from the brackish Baltic and Black Sea, and from the Rhone Delta, all heavily influenced by freshwater input, showed higher concentrations. This translates to a gradual enrichment of endospores against vegetative cells over depth, which even exceeds the prospects of Lomstein et al. (2012), who stated that endospores could be as abundant as vegetative cells in deep sediments. While accounting for ~0.1% of the vegetative cells in surface sediments, already at 100 m depth, endospores are typically more abundant than their vegetative counterparts. In deeply buried sediments metabolically quiescent cells thus clearly outnumber vegetative cells, and the potential presence of spore-like resistance forms not associated with the *Firmicutes* could even tip the balance further towards dormancy.

In order to try to provide a global estimate of endospore abundance in the marine subsurface, we followed an approach based on the estimation of vegetative cells by Kallmeyer et al. (2012). Accordingly, decrease of endospores over depth at individual sites was adjusted to a power curve, which can be described by the variables cell concentration at 1 m depth and rate of decrease. These two variables were then correlated to environmental parameters. Making use of the available global grids of these parameters, abundance of bacterial endospores in the habitable marine subseafloor could be modelled (Fig. 1). Thereby we provide evidence for a formidable community of endospores populating the marine biosphere, a community that, besides acting as time capsules or seed banks, may play an active role in the deep biosphere through selective or stochastic germination during burial.



**Figure 1** Estimated global distribution of endospores integrated over sediment depth and expressed per  $\text{km}^2$

## References

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