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ANALYSIS OF SPORE DIPICOLONIC ACID CONTENTS FOR ESTIMATING THE NUMBER OF ENDOSPORES IN SEDIMENTS

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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. Since they can be stained with fluorescent dyes they may contribute significantly to total cell counts emphasizing the need for a method for the estimation of endospores in sediments.

In the present study, dipicolinic acid (DPA), which is accumulated in the endospore core, was used to quantify spores in sediment samples from the backbarrier tidal flat of the island of Spiekeroog in the southern North Sea. For converting sediment DPA contents into endospore numbers, ten bacterial strains were examined for their DPA content per endospore. Six strains (*Bacillus* sp. G50II, *Bacillus* sp. G400 I, *Bacillus* sp. N300I, *Bacillus* sp. NA402, *Oceanibacillus* sp. NC301 and *Clostridiales* bacterium strain G100XIII) were isolated from the sediment sampling location. The other four strains comprised two well described *Bacillus* species from a culture collection (*B. megaterium* DSM 32^T and *B. subtilis ssp. subtilis* DSM 10^T) and two sulphate-reducing bacteria *Desulfosporosinus orientis* DSM 765^T and *Desulfotomaculum* sp. B2T (Sass & Cypionka, 2004). DPA contents of endospores ranged from 1.4×10^{-16} mol (*Bacillus* sp. G400I) to 1.3×10^{-15} mol (*Desulfosporosinus orientis* DSM 765^T). The observed differences in spore DPA content of the different strains apparently corresponded well with variations in spore volume (Fig. 1). Average spore volumes determined for the different strains ranged from 0.40 µm^3 to 2.43 µm^3, with spores of *Desulfotomaculum* sp. B2T being largest and having six times the volume determined for *Oceanibacillus* sp. NC301 spores. A rough correlation of DPA content and volume was indicated by linear regression using all data points, resulting in a DPA concentration of 0.46 mol DPA l^{-1} which fits well to all strains with the exception of *Desulfosporosinus orientis* DSM 765^T. The spores of the tidal flat isolates showed little variation in size and DPA content and generally contained less DPA than endospores of the two sulphate reducers or of *Bacillus megaterium*^{T}. For the tidal flat strains, an average of 2.2×10^{-16} mol DPA per spore was determined and used for conversion of sediment DPA contents into spore numbers. Estimated endospore numbers were in a range of 10^6 to 10^7 endospores g^{-1} sediment and exceeded viable counts of spores determined after oxic incubation in pasteurized MPN series

(Köpke et al., 2005) by at least three orders of magnitude, indicating that only a minor fraction of the endospores in the sediment can be detected by cultivation-dependent approaches. Since quantification on the basis of dipicolinic acid contents does neither discriminate between viable and non-viable spores nor between different physiological groups, it apparently provides a more realistic estimate of the contribution of endospores to the microbial community. For this reason, we suggest the use of DPA for determination of endospore numbers as a valuable amendment to total cell counts to reveal the importance of endospores in sedimentary microbial communities.

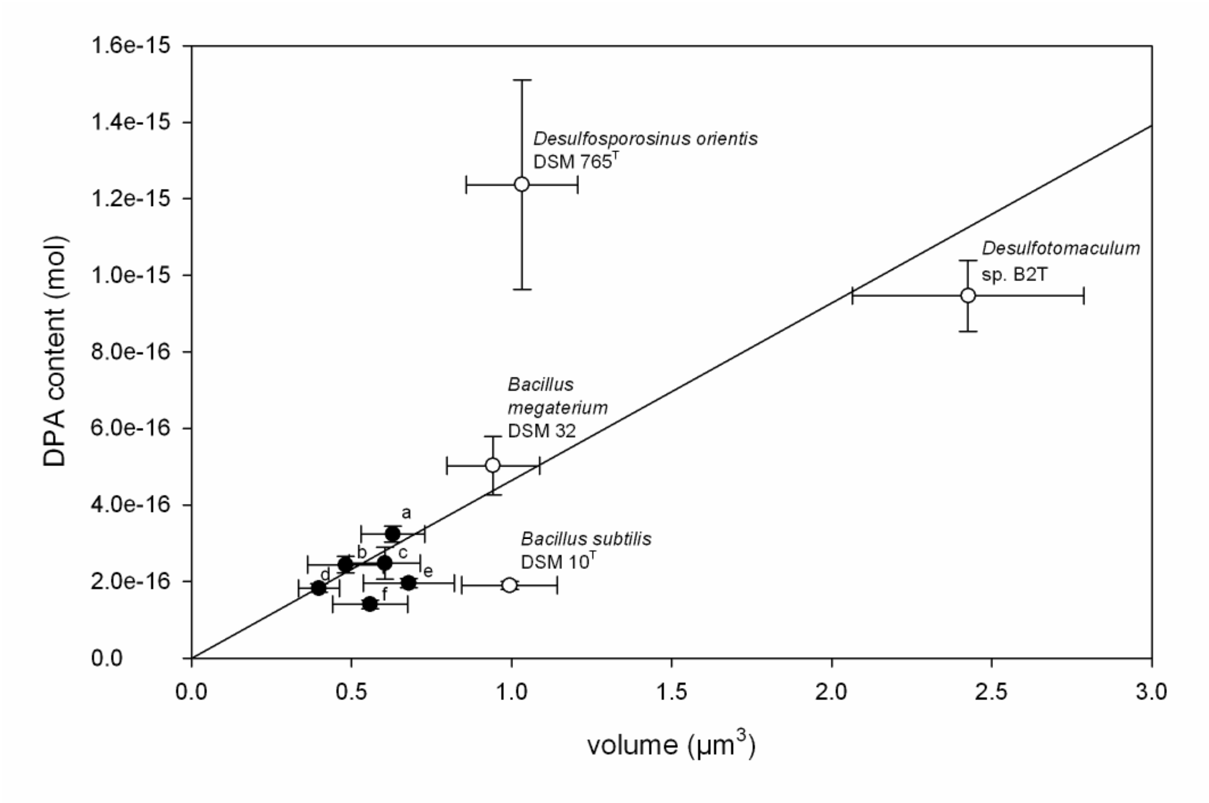

Figure 1. Plot of DPA content versus volume of the endospores analyzed in this study. The regression line (including all data points) represents 0.46 mol DPA l^{-1} spore volume and fits well to most of the strains. Black dots: tidal flat isolates a) *Bacillus* sp. NA402 b) Strain G100XIII c) *Bacillus* sp. N300I d) *Oceanibacillus* sp. NC301 e) *Bacillus* G50II f) *Bacillus* G400I. White dots: sulphate reducers and aerobic freshwater bacilli.

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