An improved dipicolinic acid (DPA) based method for detecting endospores in low-biomass sedimentary samples

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Endospore formation is a survival strategy of bacteria in the phylum Firmicutes, employed to face, e.g., low nutrient conditions or high temperatures, and is slowly being recognized as a potentially important strategy in the deep biosphere [1].

Accurate chemical detection of endospores in sediment samples has been enabled by dedicated chromatographic methods targeting the highly diagnostic biomarker dipicolinic acid (DPA), which is specific to intact endospores [1, 2]. However, extraction of this biomarker from sediments may be hampered by adsorption of DPA to the matrix, especially by the common clay minerals kaolinite and smectite.

We conducted experiments with sand, kaolinite and montmorillonite (smectite), amended with endospores from pure cultures, to develop a protocol to (i) minimize the adsorption of DPA during extraction by fine-tuning and stabilizing the pH conditions, (ii) test additives which enhance extraction yields by actively releasing DPA from the matrix, (iii) adjust the instrument conditions and setup to improve chromatographic conditions, and (iv) upscale the sample volume and concentration of extract to lower the detection limit further. Optimzed pH conditions alone resulted in a at least one order of magnitude higher sensitivity in kaolinite and smectite matrix and recovery of DPA reached ~90% compared to non-matrix extraction blanks. Along with the other improvements, this new method will enable detection of endospores in clay-rich samples from low-biomass settings.

[1] Lomstein (2012), *Nature* **484**, 101-104. [2] Fichtel (2007), *J. Microbiol. Meth.* **70**, 319-327.