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. Optimized 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.