

# DIPICOLINIC ACID AS A TRACER FOR THERMOPHILIC ENDOSPORES AND HYDROCARBON SEEPS IN DEEP WATER MARINE SEDIMENTS

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## Introduction

Understanding the sediment biogeography of dormant marine thermophilic bacterial endospores (thermospores) has the potential to assist locating and characterising working petroleum systems. The presence of thermospores in cold ocean environments suggests that distribution is governed by spore dispersal via advective hydrocarbon seepage sourced from deep hot oil reservoirs. Low abundance and endospore coat physiology mean nucleic acid based microbiological surveillance techniques have limited success for *in situ* detection of thermospores. The biomarker 2,6-pyridine dicarboxylic acid (dipicolinic acid or DPA) is specific to endospore-forming bacteria from the phylum *Firmicutes*, and constitutes a significant percentage of endospore dry weight. DPA is therefore a potential biomarker for sediment dwelling endospores, and in particular for detecting anomalies due to oil reservoir-derived thermospores. If so, DPA could have utility in locating seabed hydrocarbon seeps, however its suitability for such seabed screening has so far not been tested.

## Results

To address this possibility we established up a modified Tb<sup>3+</sup> chelation method for the analysis of DPA (Lomstein and Jørgensen, 2012) using HPLC coupled to a fluorescence detector measuring at 270 nm emission and 545 nm excitation. Sediment samples were extracted using complete digestion with acid hydrolysis. DPA distribution was assessed in deep seabed sediment samples from 97 locations in the Eastern Gulf of Mexico (Figure 1). 16S rRNA gene amplicon libraries of thermophilic spore formers from high temperature sediment incubations (Chakraborty et al., 2018) were used to assess whether DPA detection in the sediments could be associated with the presence of different thermophilic spore forming bacteria (i.e. assessing the likelihood of DPA originating from mesophilic vs thermophilic endospores). DPA concentrations were compared with geochemistry and available seep data. Additional sediment samples from along the Scotian shelf and Laurentian channel in Atlantic Canada provided both hydrocarbon positive and negative sediment cores, and enabled higher resolution down-core DPA depth profiles. Sediment cores from deeper water showed higher and more variable concentrations of DPA down core at hydrocarbon-positive stations compared to hydrocarbon-negative stations.

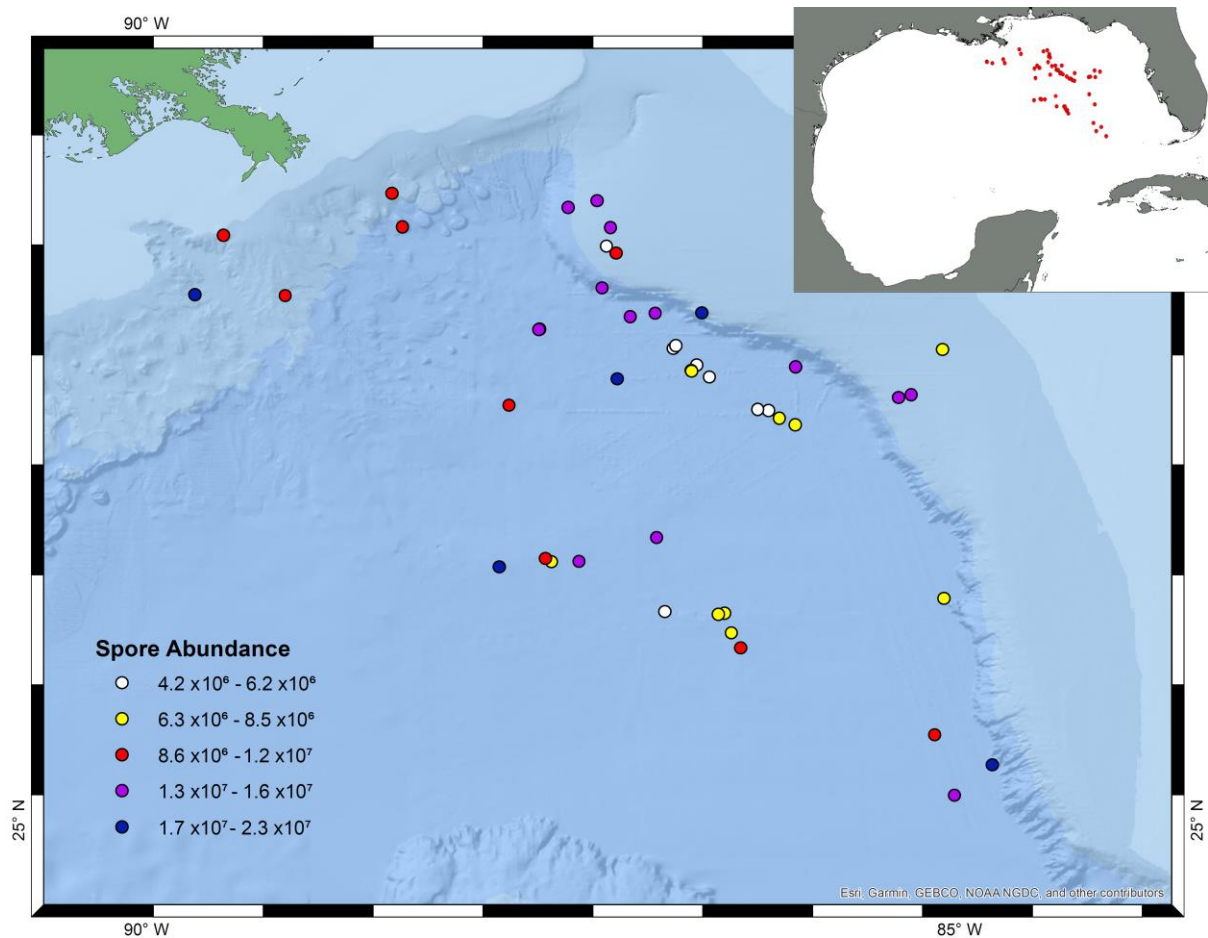


Figure 1. Distribution of endospore abundance in the Eastern Gulf of Mexico surface sediment calculated using spore specific DPA as a biomarker.

## Conclusion

The efficacy of DPA for tracing thermospores associated with hydrocarbon seeps in marine sediments has undergone preliminary assessment and we propose that DPA has potential as a biomarker for assisting in locating hydrocarbon systems in deep water marine environments, based on hydrocarbon seeps being point sources for thermophilic endospores.

## References

Chakraborty, A., Ellefson, E., Li, C., Gittins, D., Brooks, J. M., Bernard, B. B., and Hubert, C. R. J.: Thermophilic endospores associated with migrated thermogenic hydrocarbons in deep Gulf of Mexico marine sediments, *The ISME journal*, 12, 1895-1906, 2018.

Lomstein, B. A. and Jørgensen, B. B.: Pre-column liquid chromatographic determination of dipicolinic acid from bacterial endospores, *Limnol. Oceanogr. Meth.*, 10, 227-233, 2012.