

## Investigation into spore coat properties for the rapid identification of endospores in marine sediments

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### Summary

Alternative rapid analytical methods are needed to assist locating and characterising petroleum systems. The presence of dormant marine thermophilic bacterial endospores (thermospores) in cold ocean environments suggests that distribution occurs via thermal gradients but due to their impervious layered coatings, thermospores are difficult to detect. Here we present a study into the use of microscopy and staining techniques to quantify and study the behaviour of endospores in cultures, marine sediments and hydrocarbon rich materials.

### Introduction

Understanding the sediment biogeography of thermospores has the potential to assist locating and understanding fluid flow in working petroleum systems. Thermospores are genetically closely related to oil reservoir bacteria that have been found in cold surface sediments of the world's oceans. The occurrence of these 'misplaced' thermophiles in cold ocean environments suggests that distribution occurs via thermally active reservoirs, which includes those with hydrocarbon seepage where advective fluid flow transports cells and spores from the warm reservoir up into the cold ocean. However, due to low abundance and endospore coat physiology nucleic acid based techniques have had limited success in the *in situ* detection of thermospores. Alternative rapid analytical methods therefore need to be explored. In our study we investigated using the Schaeffer-Fulton (1933) (malachite green and safranin) and DAPI (4',6-diamidino-2-phenylindole) staining (Dan et al., 1971) techniques on thermospores from cultures, a natural-gas condensate, and marine sediments.

### Materials and Methods

Sediment samples from 111 locations in the Eastern Gulf of Mexico (100 to 3300 m water depth; 6 to 600 km apart) were incubated at high temperature, followed by construction of 16S rRNA gene amplicon libraries (V3-V4 region; Illumina MiSeq) revealing enrichment of different thermospore species. A sulfate-reducing bacterium from site EGM080 was purified and classified based on its rRNA gene sequence as *Desulfotomaculum geothermicum*. Prior to thermospore staining the culture was kept in the stationary/decline phase for 16 weeks to promote sporulation. A natural-gas condensate from offshore east coast Canada was also used to investigate the behaviour of spores in oil. Samples of *D. geothermicum*, source marine sediment and the natural-gas condensate were fixed using paraformaldehyde, dried and stained using the Schaeffer-Fulton (1933) method and analysed using brightfield microscopy. Samples treated with DAPI were analysed using fluorescence microscopy and untreated stained slides were observed under phase contrast microscopy.

## Examples

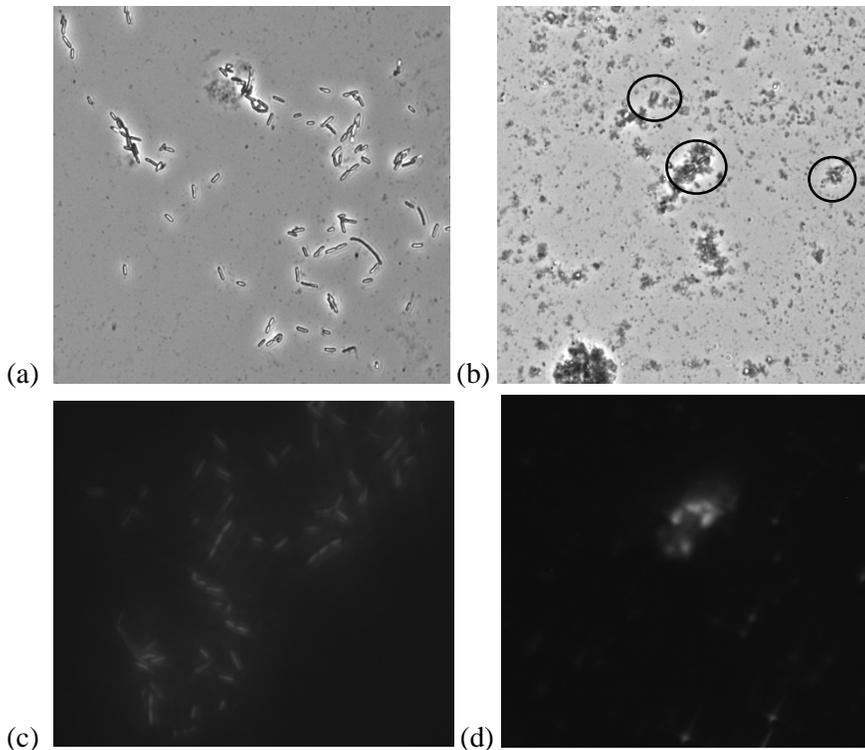


Figure 1. (a) Phase contrast microscopy of *Desulfotomaculum geothermicum* spores. Brightfield microscopy of Schaeffer-Fulton treated samples resulted in bright green stained spore cells. No red stained vegetative cells were observed. (b) *D. geothermicum* spores observed in a spiked sediment sample. (c) Fluorescence microscopy of *D. geothermicum* spores treated with DAPI. (d) DAPI treated spiked sediment sample.

## Conclusions

Thermospores in pure culture were identified using phase contrast but were difficult to observe in the sediment sample due to particle aggregation. The Schaeffer-Fulton technique aided endospore identification in a complex sediment sample matrix as spores were stained bright green, and also revealed that there were only spores and no (red stained) vegetative cells in the sample. Treatment with DAPI gave dull fluorescing cells but did provide insight into the behaviour of spores in sediment suspensions. Spores in the culture medium and oil were free floating (Schaeffer-Fulton technique) but in the sediment suspension they were only observed attached to aggregated fluorescing material (DAPI). Further investigation into thermospore association with bioparticles could facilitate our understanding of the passive dispersal of spores in marine environments.

## References

- (1) Schaeffer A, B., Fulton, M, D. (1933) A simplified method of staining endospores. *Science.*, 77, p. 194
- (2) Dann, O., Bergen, G., Demant, E., Vol, G. (1971) Trypanosomicidal diamidines of 2-phenylbenzofuran, 2-phenylindene, and 2-phenylindole. *Liebigs Ann. Chem.*, 749, p. 68