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13. Data Report: Microbiological AODC and CARD-FISH Analysis of Black Shale Samples from the Demerara Rise, ODP Leg 207[1]

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INTRODUCTION

Subseafloor sediments harbor over half of all prokaryotic cells on Earth (Whitman et al., 1998). This immense number is calculated from numerous microscopic acridine orange direct counts (AODCs) conducted on sediment cores drilled during the Ocean Drilling Program (ODP) (Parkes et al., 1994, 2000). Because these counts cannot differentiate between living and inactive or even dead cells (Kepner and Pratt, 1994; Morita, 1997), the population size of living microorganisms has recently been enumerated for ODP Leg 201 sediment samples from the equatorial Pacific and the Peru margin using ribosomal ribonucleic acid targeting catalyzed reporter deposition—fluorescence in situ hybridization (CARD-FISH) (Schippers et al., 2005). A large fraction of the subseafloor prokaryotes were alive, even in very old (16 Ma) and deep (>400 m) sediments. In this study, black shale samples from the Demerara Rise (Erbacher, Mosher, Malone, et al., 2004) were analyzed using AODC and CARD-FISH to find out if black shales also harbor microorganisms.

MATERIALS AND METHODS

Whole-round core (WRC) samples from ODP Leg 207 were shipped in anaerobic bags on ice to the Federal Institute for Geosciences and Natural Resources (BGR) in Hannover, Germany. Within 3 months of sampling, subsamples were fixed for AODC and CARD-FISH analysis.

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After removal of the ~1-cm contaminated surface layer with a sterile scalpel, a subsample from the centermost part of each WRC was taken with either a sterile scalpel or a sterile cut syringe. Immediately, 0.5 cm^3 of material was fixed and stored in ethanol:phosphate buffer solution (1:1) at –20°C until further analysis (Ravenschlag et al., 2001). CARD-FISH was applied on filters following a previously described protocol for marine bacteria (Pernthaler et al., 2002; Schippers et al., 2005). For each sample, the filter was cut into sections that were used for hybridization, targeting archaea (probe ARCH915), bacteria (probe EUB338), or no cells (probe NON338 as negative control) (Ravenschlag et al., 2001).

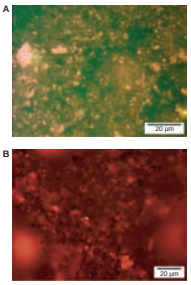
RESULTS AND CONCLUSIONS

The results of AODC and CARD-FISH analysis of black shale and sediment reference samples are shown in Figure **F1** and Table **T1.** Prokaryotes could be detected with AODC as well as with CARD-FISH in 2 of 13 black shale samples but not in the 5 reference sediment samples. The number of total prokaryotes and living bacteria in the two black shale samples are comparable to those found for Leg 201 sediment samples from the equatorial Pacific and the Peru margin (D´Hondt, Jørgensen, Miller, et al., 2003; D´Hondt et al., 2004; Schippers et al., 2005). The CARD-FISH numbers represent a minimum of the total living bacteria because living cells with very low activity and ribosome contents were probably not detected. A few living cells of archaea could be detected, but their number was too low to be quantified using CARD-FISH.

The absence of prokaryotes in most of the black shale and all reference sediment samples indicates that the highly compacted Leg 207 sediment layers studied here do not provide suitable conditions for a thriving microbial community (in contrast to Leg 201 sediments, for example). Bulk densities ranged from ~1.5 to ~2.1 g/cm^3 for Leg 207 samples and from ~1.2 to ~1.8 g/cm^3 for Leg 201 sediments. Porosities were always <70% for Leg 207 samples studied here but showed values of >75% for all Leg 201 sediments. However, the occurrence of prokaryotes in at least two Leg 207 black shale samples suggests a patchy distribution of the cells probably living in larger pores or cracks. Because contamination tests were not carried out during Leg 207 as described for Leg 201 (D´Hondt, Jørgensen, Miller, et al., 2003), contamination of the two black shale samples can not be excluded, but it is unlikely because of the high numbers of prokaryotes detected.

The interstitial water geochemistry of Leg 207 sediments indicates microbial activity at the top of the black shale sequence. At all sites (except Site 1261), sulfate and ammonium gradients are essentially linear from the sediment/seawater interface to the top of the black shale sequence (Erbacher, Mosher, Malone, et al., 2004). Unfortunately, we have not received samples from these depth intervals to verify the presumably high abundance of prokaryotes there.

F1. Detection of prokaryotes, p. 5.



T1. Microbiological analysis, p. 6.

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Figure F1. Detection of prokaryotes in two black shale samples (Sections 207-1258B-51R-2 and 207-1259C-17R-1). **A.** Total prokaryotes stained with acridine orange (green). **B.** A living bacterium detected using CARD-FISH with the probe.

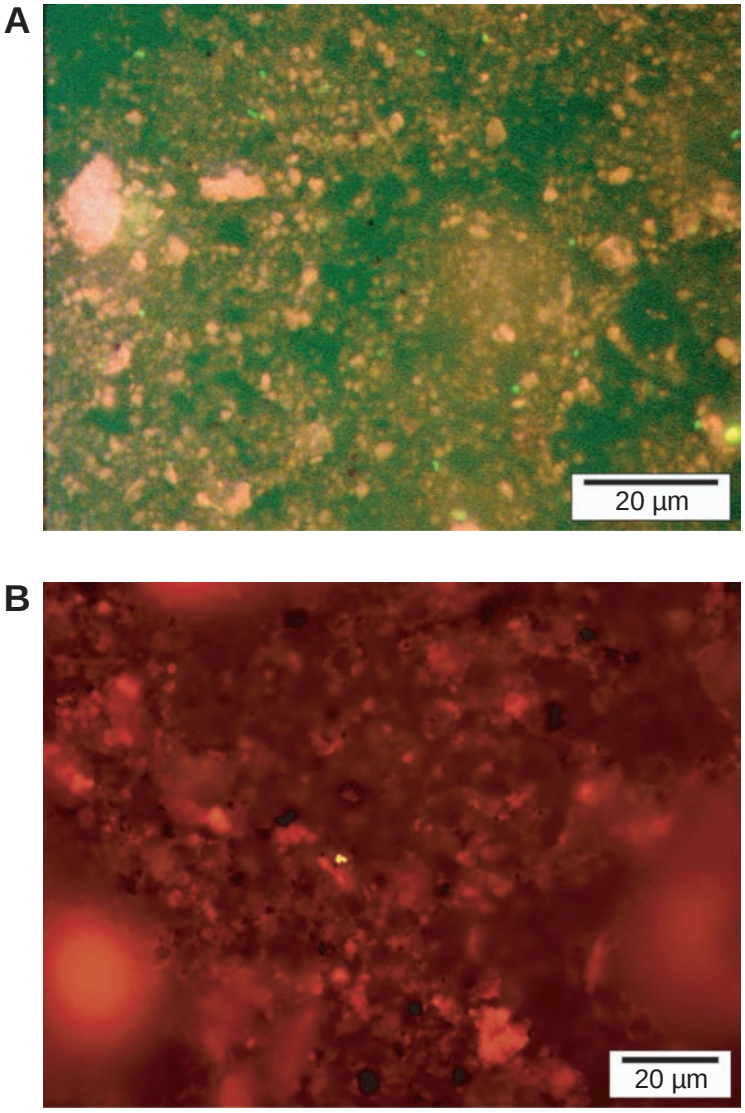

Table T1. Results of the microbiological analysis of black shale and reference samples of the Demerara Rise, ODP Leg 207.

Core	Depth (mbsf)	Black shale	AODC, total prokaryotes (N/cm^3)	CARD-FISH: Living bacteria (N/cm^3)	CARD-FISH: Living archaea
207-1257C-					
3R	105.7	No	ND	ND	ND
12R	190.1	Yes	ND	ND	ND
13R	200.2	Yes	ND	ND	ND
16R	233.8	No	ND	ND	ND
207-1258B-					
42R	385.1	No	ND	ND	ND
45R	399.2	Yes	ND	ND	ND
46R	403.7	Yes	ND	ND	ND
51R	427.8	Yes	1.9×10^7	3.0×10^5	Detected
52R	434.0	Yes	ND	ND	ND
54R	444.5	Yes	ND	ND	ND
55R	448.5	Yes	ND	ND	ND
207-1259C-					
8R	444.4	No	ND	ND	ND
11R	494.5	Yes	ND	ND	ND
16R	525.6	Yes	ND	ND	ND
17R	529.2	Yes	8.7×10^6	4.7×10^5	Detected
207-1261B-					
4R	553.7	No	ND	ND	ND
6R	569.9	Yes	ND	ND	ND
13R	643.0	Yes	ND	ND	ND

Notes: AODC = acridine orange direct count, CARD-FISH = catalyzed reporter deposition–fluorescence in situ hybridization, ND = not detected. The detection limit was 5×10^4 cells/cm^3 for minimum 100 fields of view observed.