30. A SHORT REPORT ON MICROBIOLOGY OF SEDIMENTS FROM DEEP SEA DRILLING PROJECT HOLES 415, 415A, AND 416A

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The aim of this work was to determine whether viable cells of methane-forming bacteria are present in the sediments of Holes 415, 415A, and 416A, and if so, to determine their number and the intensity of methane formation. The sediment samples were taken in sterile polyethylene packets and kept frozen until they were analyzed. E. M. Galimov of the Vernadsky Institute of Geochemistry and Analytical Chemistry, U.S.S.R. Academy of Sciences, Moscow, a geochemist during DSDP Leg 50, provided the samples for our study.

METHODS

Analysis for viable cells of methane-forming bacteria was performed by inoculation of a nutrient medium with a 10-fold dilution of sediments (Belyaev, 1974), using the anaerobic technique described by Hungate (1969). The maximum weight of the inoculum was 1 gram. The culture was grown in 15-ml glass flasks on Bryant's medium (Bryant and Robinson, 1961). It contained 0.5 per cent sodium acetate, 3 per cent NaCl, and 0.5 per cent vitamin solution (Bryant et al., 1971) instead of rumen fluid and carbohydrates. The gas phase consisted of 75 per cent H_2 and 25 per cent CO_2. The possible development of methane-forming bacteria was monitored chromatographically. Incubation time was six months. Psychrophylic, mesophylic, and thermophylic types of methane-forming bacteria have been found in ocean sediments taken as deep as 1600 meters below the sea floor; thus, the incubation was performed at 2 °C, 28 °C, and 45 °C.

The intensity of methane formation by modern microbiota was determined using the radioisotopic method of Ivanov et al. (1976). Radioisotopes of ^{14}C were introduced in the form of $NaHCO_3$, at activity of 0.45 μc per 10 grams of sediment. The samples were exposed for six months. The radioactivity of the methane formed was registered on a scintillation counter, with previous burning of methane to CO_2.

RESULTS AND DISCUSSION

The results of investigations, given in Table 1, show that methane-forming bacteria did not develop in samples inoculated in nutrient medium. On the basis of these data, we conclude that viable cells of methane-forming bacteria are either absent or present in numbers fewer than one cell per gram of the analyzed sediment.

The data shown in Table 2 indicate that no methane is being produced by microbiota in the sediment analyzed. Note that the radioisotopic method allows us to record an intensity of methane formation up to 10^{-7} ml CH_4/1 kg of sediment/day.

The intensity of methane formation in ocean sediments per one viable cell is 2–110 $\times$ 10^{-11} ml CH_4/day (Belyaev and Laurinavichus, 1977). Thus, the sensitivity of the radioisotopic method was sufficient to record the process performed by one cell in 10 grams of sediment.

Unfortunately our microbiological and radioisotopic experiments were carried out 2 to 5 months following

TABLE 1
Presence of Viable Cells of Methane-Forming Bacteria in Sediments of Holes 415, 415A, and 416A

Sample (Interval in cm)	Sub-Bottom Depth (m)	Number of Methane-Forming Bacteria (cells/gram) 2°C	28°C	45°C
415-1-5, 37-62	0.0-7.5	0	0	–[a]
415-2-1, 46-72	74.0-83.5	0	0	–
415-4-5, 117-135	207.0-216.5	0	0	–
415A-5-1, 101-126	443.0-452.5	–	0	–
415A-10-2, 110-136	709.0-718.5	–	0	0
415A-14-2, 6-31	1032.0-1041.5	–	0	0
416A-1-1, 115-140	146.0-155.5	0	0	–
416-3-3, 60-87	450.0-459.5	–	0	–
416A-6-3, 29-75	887.0-896.5	–	0	0
416A-22-3, 68-86	1299.5-1309.1	–	0	0
416A-50-2, 31-81	1548.6-1558.0	–	0	0

[a]Sample not analyzed (–).

TABLE 2
Intensity of Methane Formation by Living Microbiota in Sediments From Holes 415, 415A, and 416A

Sample (Interval in cm)	Sub-Bottom Depth (m)	Intensity of Methane Formation (ml CH_4/kg/day) 2°C	28°C	45°C
415-1-5, 37-62	0.0-7.5	0	–	–[a]
415-2-1, 46-72	74.0-83.5	0	–	–
415-4-5, 117-135	207.0-216.5	0	–	–
415A-5-1, 101-126	443.0-452.5	–	–	–
415A-10-2, 110-136	709.0-718.5	–	0	–
415A-14-2, 6-31	1032.0-1041.5	–	–	0
416A-1-1, 115-140	146.0-155.5	–	–	–
416A-3-3, 60-87	450.0-459.5	–	–	–
416A-6-3, 29-75	887.0-896.5	–	0	–
416A-22-3, 68-86	1299.5-1309.1	–	–	0
416A-50-2, 31-81	1548.6-1558.0	–	–	0

[a]Sample not analyzed (–).

recovery of the core samples; thus we cannot be certain that methane-forming organisms were not initially present in the sediment.

CONCLUSIONS

1. Viable cells of methane-forming bacteria are not present in the tested samples.
2. No methane formation by living microbiota was detected in the tested samples.

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