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NOTE

Thermophilic bacterial activity in a deep-sea sediment from the Pacific Ocean

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ABSTRACT: Thermophilic bacterial activity was detected in a deep-sea sediment sample from the South Pacific Ocean at 12° S, 135° W, an area of the seafloor distant from known hydrothermal venting. Incubation of sediments amended with ^{14}C-glutamate indicated maximal respiration (evolution of $^{14}CO_2$) and assimilation (incorporation of ^{14}C into acid-precipitated macromolecules) of substrate at 52°C, relative to 4 and 22°C. A parallel experiment at another site (2° S, 140° W) yielded no evidence of thermophily. Thermophilic bacteria may be deposited in deep-sea sediments following their long-distance dispersal from hydrothermal vents (e.g. the East Pacific Rise and other sites), via either continuous venting or formation of megaplumes.

KEY WORDS: Deep sea · Thermophilic bacteria · Hydrothermal venting · Dispersal

Most of the ocean's sedimentary environment is cold, given that deep-water (>1000 m) temperatures typically range between 0 and 4°C (Knauss 1978). Indigenous deep-sea bacteria, therefore, generally are considered adapted to grow at low temperatures ('psychrophilic') (e.g. Zobell & Morita 1959, Jannasch & Taylor 1984). Nonetheless, thermophilic bacteria have been isolated from deep-sea sediment (Bartholomew & Rittenberg 1949, Bartholomew & Paik 1966). The presence of these so-called 'alien bacteria' (Bartholomew & Paik 1966) was enigmatic prior to the discovery in the late 1970s of hydrothermal vents along the mid-ocean ridge. Indeed, both aerobic (Marteinsson et al. 1995) and anaerobic (e.g. Huber et al. 1989) thermophilic bacteria have been isolated from deep-sea hydrothermal vents. Furthermore, thermophilic bacterial activity has been reported in water emanating from deep-sea hydrothermal plumes (review in Karl 1995). There have been no published accounts, however, of thermophilic activity in deep-sea sediments distant from hydrothermal vents. This note is the first report of such activity and suggests hydrothermal venting and deep-water circulation may facilitate long-distance transport of thermophiles.

Samples were collected during Cruise TT013 (October to December 1992) of the Joint Global Ocean Flux Study in the Equatorial Pacific. Because of time and material constraints, tests for thermophily were conducted only at Stn 10 (12.00° S, 134.95° W, depth = 4280 m) and Stn 23 (1.87° S, 139.80° W, depth = 4380 m). These stations are located on flat, sediment-covered abyssal plains, many hundreds of km distant from any known hydrothermal activity. Virtually undisturbed samples of sediment were collected using a multicorer (Barnett et al. 1984). Within 5 min of the multicorer's arrival on deck, the tube containing sediment for analysis was transferred to a cold room. Overlying water was carefully siphoned off, the sediment was extruded, and samples were taken to determine carbon turnover at simulated deep-sea temperature and pressure (Dobbs & Selph unpubl.). As a very modest compliment to this primary study, sediment from the uppermost horizon (0 to 5 mm) was examined for thermophilic activity.

Microbial utilization of carbon was evaluated using the method developed by Deming (1993). Sediment was homogenized in a sterile Petri dish, then diluted 12-fold with filtered (0.2 µm) seawater. Four ml of the slurry were drawn into a plastic 5 ml syringe already containing 1.0 ml of radiolabeled glutamic acid in a solution of filter-sterilized seawater (Amersham, L-[U-^{14}C]glutamic acid, specific activity 266 mCi mmol^{-1}; final concentration ≤1.5 ng ml^{-1}). A sterile needle was affixed to the Luer end of the syringe and inserted into a small piece of rubber. Groups of syringes were placed into plastic bags, which then were sealed and incubated in water baths for 24 h at 4, 22, or 52°C. The warmest treatment was achieved by adding hot water every 2 h to an insulated container; therefore,

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temperature varied over time (mean of 52°C, range over 2 h was 47 to 57°C). Temperature was held nearly constant (±1°C) at the 2 lower temperatures. Samples were mixed by shaking them every 2 h. Poisoned sediments (formalin, final concentration 8 ml ml^{-1}) served as controls for abiotic uptake of radioactivity. All treatments and controls were prepared in duplicate.

To terminate the incubations, the sediment slurries in the syringes were injected into serum bottles containing acid (1.0 M HCl). $^{14}CO_2$ was collected on strips of paper containing phenethylamine and radioassayed (Hobbie & Crawford 1969, Iverson et al. 1976) using Optima Gold (Packard) as the liquid scintillation fluor. Macromolecules were precipitated with cold trichloroacetic acid (10% final concentration, w/v), collected on GF/F filters, and radioassayed as above. Background counts were subtracted from sample counts before counts per minute (CPM) were converted to disintegrations per minute (DPM) using the external-standards channels ratio. Samples were counted for 20 min or until counting error was ≤2%. Results of the $^{14}CO_2$ assay represent respiration, those of the ^{14}C-macromolecule assay, assimilation. Within-station comparisons were made to test treatment effects (Deming 1993).

A thermophilic response to the provision of ^{14}C-glutamic acid was evident at Stn 10, where bacterial respiration was 3.6 and 13.2 times greater at 52°C than at 4 and 22°C, respectively (Fig. 1A). A very similar pattern emerged with respect to assimilation; values at 52°C were 2.1 and 9.9 times greater than at 4 and 22°C, respectively. On the other hand, a thermophilic response was not detected at Stn 23, where respiration and assimilation were 17 to 52 times greater at 4 and 23°C than at 52°C (Fig. 1B). Values for assimilation controls at Stn 10 were slightly more than twice those at Stn 23, presumably related to the >2-fold difference in ^{14}C-glutamate concentrations used. We cannot account, however, for the incongruous respiration controls at Stn 10, but note the values were independent of temperature.

Because the intent of these experiments merely was to test for a thermophilic 'treatment effect' (Deming 1993), we did not incorporate into this analysis interstation differences in glutamate specific activity, bacterial numbers, or bulk sediment properties. Furthermore, our experimental design precluded determination of a barophilic response, although there exists a significant barophilic component in deep-sea, sedimentary bacterial communities (e.g. Zobell & Morita 1959, Jannasch & Taylor 1984, Deming & Colwell 1985). Finally, a 24 h incubation could measure metabolic activity only of bacteria already active or those reactivated by sample processing.

The thermophilic organisms in these experiments were heterotrophic, or at least facultatively so; inherent in the assay is their ability to metabolize glutamate. Also, they functioned in the presence of oxygen; no precautions were taken to limit gas diffusion or to provide reducing conditions. Furthermore, they were not obligate barophiles; all incubations were conducted at 1 atmosphere pressure. As to their degree of thermophily, this study's highest temperature treatment (52°C) approximated the threshold at which prokaryotic thermophily is considered to begin, i.e. 55 to 60°C (Brock 1985) and 60°C (Baross & Deming 1995). Thus, although the bacterial activity reported here arguably is thermophilic, it is at the lower end of the operational definition.

There is no evidence the thermophilic effect at Stn 10 was produced by a eurythermal population(s) of

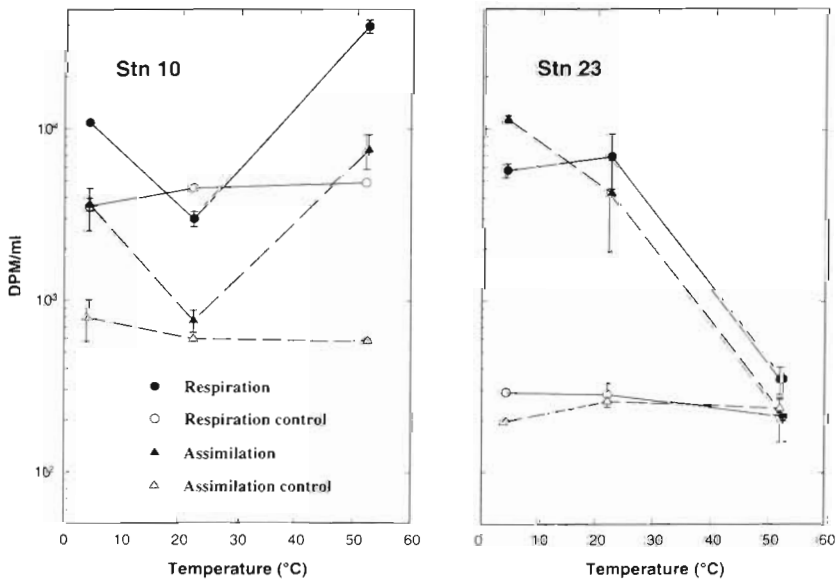

Fig. 1 Respiration and assimilation of ^{14}C-glutamate in deep-sea sediments. (A) Stn 10 (12.00° S, 134.95° W) depth = 4280 m; (B) Stn 23 (1.87° S, 139.80° W) depth = 4380 m. Data are $\log_{10}$ disintegrations min^{-1} (DPM) per ml of sediment (Stn 10: 1 ml sediment = 0.279 ± 0.019 g dry mass; Stn 23: 1 ml sediment = 0.345 ± 0.016 g dry mass; mean ± 1 SD, n = 4). Plotted values represent mean DPM ml^{-1} ± 1 SD (n = 2). When not visible, the standard deviation is less than the size of the symbol. The specific activity of ^{14}C-glutamate used at Stn 10 was 5.8×10^3 DPM ml^{-1}, equivalent to a glutamate concentration of 1.5 ng ml^{-1}; corresponding values at Stn 23 were 2.4×10^3 DPM ml^{-1} and 0.6 ng ml^{-1}. Control values represent samples poisoned with formalin

bacteria. Respiration and assimilation were greater at 4 and 52°C than at 22°C, suggesting psychrophilic and thermophilic populations were more metabolically active than mesophilic bacteria, at least with respect to glutamate as a substrate.

These experiments were not designed to detect extreme thermophiles, also called hyperthermophiles (e.g. Baross & Deming 1995). This group of organisms, with optimal growth temperatures of 80°C or higher, probably contributed little, if anything, to these results. Nearly all marine hyperthermophiles are strict anaerobes (Baross & Deming 1995), and although they can survive for years in the laboratory in the presence of oxygen if kept cold (Stetter 1995), they would not have been metabolically active under the conditions described here.

Given ca 450 km distance to the nearest inhabited island, it is most unlikely that terrestrial sources of thermophilic cells figured significantly in these results. While the possibility exists that overboard disposal from ships may contribute minimally to thermophiles in deep-sea sediments, a more intuitive source of such bacteria is the vast expanse of hydrothermal vents associated with the mid-ocean ridge, ridge flanks, and geophysical 'hot spots' in the Pacific Ocean. The flux of dissolved and particulate materials from these vents imparts on the emanating water not only a distinctive chemical signature, but in many systems enriches its microbial components as well (review in Winn et al. 1995). In addition to continuous but variable steady-state venting, so-called 'megaplumes' are formed through rapid and massive injection of hydrothermal fluids (and their microbial constituents) into the overlying water column (Baker et al. 1987, Cann & Strens 1987).

Whatever the method of venting, the warm water so released rises quickly and forms a neutrally buoyant plume detectable hundreds, even thousands, of kilometers downstream (e.g. Lupton & Craig 1981). Bacterial numbers and biomass are elevated in hydrothermal plumes, due in part to growth of cells utilizing the plumes' organic and inorganic energy sources (Winn et al. 1995). Particle-associated cells not digested by predators or lysed by viruses eventually will fall to the sediments. Dymond & Roth (1988) estimated the residence time for particles in a plume to be on the order of 100 d. It seems reasonable, then, to hypothesize a diminishing community of hydrothermal-vent bacteria in deep-sea sediments downstream of vents, analogous to the trail of microbial and geochemical signatures in the water column (e.g. Lupton & Craig 1981, Winn et al. 1995).

Therefore, we suggest plume-transported and plume-generated microbial biomass was the source material for the thermophilic activity detected at Stn 10. While the precise point of origin cannot be known, inspection of general circulation patterns do not rule out considering the East Pacific Rise (EPR), more than 2000 km to the east. We note the plume of the EPR's ^{3}He-anomaly extends directly over Stn 10 and is discernible to the west for hundreds more km (Lupton & Craig 1981, Urabe et al. 1995). However, given the relative proximity of many recently discovered mid-plate hot spots in the South Pacific (Fornari et al. 1987), closer hydrothermal systems are more plausible sources.

If the dispersal of plumes and megaplumes is indeed so extensive, then why was there no evidence of thermophily at Stn 23? The answer is suggested by comparison of sediment characteristics at the 2 sites. Sediments at Stn 10 are between 20 and 40% hydrothermal in origin and composed principally of nonbiogenic particles, whereas those at Stn 23 are predominantly (80%) biogenic particles of calcium carbonate (Leinen & Stakes 1979). If thermophiles are attached to hydrothermal particles, then they would be more abundant at Stn 10. Furthermore, particle-deposition rates at Stn 23 are more than 3 times greater than at Stn 10 (Honjo et al. 1995), consistent with relatively high levels of circum-equatorial primary productivity. Therefore, any thermophiles deposited at the sediment-water interface near the equator would be 'diluted' relative to their concentration at Stn 10.

Long-distance advective dispersal of hydrothermal microorganisms may account in part for the presence of certain *Archaea* in the water column. For example, Huber et al. (1990) reported a minimum hyperthermophile concentration of 10^6 l^{-1} within the hydrothermal plume of Macdonald Seamount, which erupted only 40 m below the ocean's surface. It seems improbable, however, that thermophilic *Archaea* are active members of the bacterioplankton community, as suggested for other *Archaea* detected in the water column (DeLong 1992, Massana et al. 1997). Instead, thermophiles likely are metabolically dormant (or dying) and merely in transit following their discharge from a hydrothermal vent.

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