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Recent studies on bacterial populations and processes in subseafloor sediments: A review

R. John Parkes · Barry A. Cragg · Peter Wellsbury

Abstract Indirect chemical evidence suggests the presence of bacteria in subseafloor sediments; however, until recently this had not been confirmed by comprehensive microbiological studies, applied either to a range of depth intervals at a single site or to a global range of sites. This paper summarizes the results of a detailed study of bacterial populations and activities at 14 sites, sampled by the Ocean Drilling Program since 1986, in the Pacific and Atlantic Oceans and the Mediterranean Sea. Surprisingly large bacterial populations are present in subsurface marine sediments, and although bacteria generally decrease with increasing depth, bacterial biomass to the average ocean sediment depth (500 m) is equivalent to about 10% of the total surface biosphere. Populations are significantly correlated with sediment depth but much less so with age or porosity. Bacterial populations and activity can increase where geochemical conditions change in deeper layers, such as brine incursion or the presence of thermogenic methane. This situation emphatically demonstrates the viability of bacteria in deep sediments, which is further supported by (1) the presence of intact prokaryotic DNA with high molecular weight, (2) some unique 16S rRNA gene sequences, (3) a range of different culturable bacteria and bacteria activities, and (4) isolated pure cultures adapted for deep sediment conditions. An extreme example of elevated subsurface bacterial populations occurs in gas-hydrate sediments, where bacterial processes are stimulated around the base of the deep gas-hydrate zone. For some activities, such as methanogenesis, anaerobic methane oxidation, and acetate metabolism, potential rates in the subsurface are greater than those near the sediment surface.

Subsurface bacteria also occur in hydrothermal sediments with large temperature gradients (up to 12 °C/m) and with population numbers similar to non-hydrothermal sites at temperatures from psychrophilic to mesophilic. At greater depths and temperatures, populations decline rapidly, but they are still significant up to hyperthermophilic temperatures and are even stimulated by subsurface seawater flow. These results suggest that temperature alone does not limit bacteria in non-hydrothermal sediments until about 4 km, and evidence exists that bacterial processes may even be sustained by interaction with thermogenic processes as temperatures increase during deep burial.

Experiments demonstrate that in the presence of readily degradable organic substrates, actively growing bacteria can move faster than sediment deposition; hence, these bacteria are not necessarily trapped and buried. However, bacterial growth decreases with depth to such an extent that subsurface bacteria would not be able to keep up with sedimentation rate and hence would be buried. In some circumstances, such as in sapropel layers with high organic matter in the Mediterranean, bacteria may be buried within a specific deposition horizon. Subsurface bacteria can utilize old and recalcitrant organic matter, but only very slowly, and they seem to have a strategy of high biomass and low growth rate, commensurate with their geological habitat of generally low energy flux.

Résumé Des données chimiques indirectes laissent penser qu'il existe des bactéries dans les sédiments des fonds sous-marins; cependant, jusqu'à présent ceci n'avait pas été confirmé par des études microbiologiques complétes, qu'elles soient appliquées à une gamme d'intervalles de profondeur en un même site ou sur un ensemble de sites. Ce papier synthétise les résultats d'une étude détaillée de populations bactériennes et de leur activité en 14 sites échantillonnés au cours des campagnes ODP (Ocean Drilling Programme) depuis 1986, dans le Pacifique, dans l'Atlantique et en Méditerranée. Des populations bactériennes étonnamment importantes sont présentes dans la profondeur des sédiments sous-marins; bien qu'elles diminuent généralement avec la profondeur, la biomasse bactérienne pour une profondeur moyenne de sédiments océaniques (500 m) équivaut à environ 10% de la biosphére bactérienne totale de la surface. Les populations sont corrélées de façon signi-

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R. John Parkes (✉) · Barry A. Cragg · Peter Wellsbury
Department of Earth Sciences, University of Bristol,
Wills Memorial Building, Queens Road, Bristol BS8 1RJ, UK
Fax: +44-117-9253385
e-mail: J.Parkes@bris.ac.uk

ficative à la profondeur du sédiment, mais beaucoup moins à son âge ou à sa porosité. Les populations bactériennes et leur activité peuvent augmenter lorsque les conditions géochimiques changent dans les couches plus profondes, par exemple avec une intrusion d'eau salée ou avec la présence de méthane d'origine thermale. Cette situation démontre trés clairement la survie des bactéries dans les sédiments profonds, ce qui est confirmé en outre par (1) la présence d'ADN procaryote intact, à masse moléculaire élevée, (2) quelques séquences uniques de gênes 16S rRNA, (3) une gamme de différentes bactéries cultivables et d'activités bactériennes, et (4) des cultures pures isolées adaptées aux conditions régnant dans les sédiments profonds. Un exemple extrême de populations bactériennes souterraines est connu dans les sédiments à hydrates gazeux, dans lesquels les processus bactériens sont stimulés aux environs de la base de la zone profonde d'hydrate gazeux. Pour certaines activités, telles que la méthanogenése, l'oxydation anaérobie du méthane et le métabolisme de l'acétate, les taux de potentiel dans le milieu souterrain sont plus élevés que ceux existant prés de la surface des sédiments.

Les bactéries souterraines existent aussi dans les sédiments d'origine hydrothermale où régnent des gradients de température élevés (jusqu'à 12 °C/m) et où sont présentes des populations semblables en nombre à celles des sites non hydrothermaux, à températures psychrophiles à mésophiles. A de plus grandes profondeurs et à des températures plus élevées, les populations diminuent trés rapidement, mais elles sont encore significativement présentes jusqu'aux températures hyperthermophiles et sont même stimulées par l'écoulement d'eau marine souterraine. Ces résultats laissent penser que la température seule ne limite pas les bactéries dans les sédiments non hydrothermaux jusqu'à environ 4 km; selon certains indices, les processus bactériens peuvent même être entretenus par des interactions avec les processus liés à la température lorsque les températures augmentent au cours de l'enfouissement profond du sédiment.

Ces expériences démontrent qu'en présence de substrats organiques dégradables les bactéries à croissance active peuvent se déplacer plus rapidement que le sédiment se dépose; ainsi ces bactéries ne sont pas nécessairement piégées et enfouies. Cependant, la croissance bactérienne décroît jusqu'à une profondeur à laquelle les bactéries souterraines ne seraient plus capables de garder le dessus sur le taux de sédimentation et finiraient donc par être enfouies. Dans certaines circonstances, comme dans les couches à sapropel à forte teneur en matiére organique de la Méditerranée, les bactéries peuvent être enfouies dans une couche particuliére. Certaines bactéries souterraines peuvent utiliser une matiére organique ancienne et résistante, mais seulement trés lentement; elles semblent posséder une stratégie de biomasse élevée et de faible taux de croissance, adaptée à leur habitat géologique à flux d'énergie généralement faible.

Resumen La presencia de bacterias en los sedimentos que forman el subsuelo marino, sugerida indirectamente por evidencias químicas, no había sido confirmada hasta muy recientemente por estudios microbiológicos globales. En este artículo se presentan los resultados de un estudio de detalle realizado en 14 poblaciones bacterianas dentro del Programa de Perforación Oceánica (Ocean Drilling Program). Las muestras se tomaron desde 1986 en los océanos Pacífico y Atlántico y en el Mar Mediterráneo. Sorprendentemente se detectaron grandes poblaciones bacterianas en los sedimentos marinos y, aunque el número de bacterias decrece con la profundidad, la biomasa bacteriana registrada hasta una profundidad media de los sedimentos oceánicos (500 m) supone alrededor del 10% del total presente en la biosfera superficial. Las poblaciones están correlacionadas significativamente con la profundidad, pero no con la porosidad o la edad de los sedimentos. Las poblaciones bacterianas y su actividad pueden incrementarse en las capas profundas, donde varían las condiciones geoquímicas por intrusión de salmueras o por la presencia de metano termogénico. Esto demuestra que es factible la presencia de bacterias en sedimentos profundos, lo que además se sustenta por (1) la presencia de DNA procariótico intacto de alto peso molecular, (2) algunas cadenas genéticas de 16S rRNA únicas, (3) diversidad en los tipos de bacterias y en la actividad bacteriana y (4) aparición de bacterias en cultivos aislados sometidos a las condiciones de los sedimentos. Un ejemplo extremo de grandes poblaciones bacterianas lo encontramos en sedimentos gas-hidratados, los cuales estimulan los procesos bacterianos.

Las bacterias también pueden aparecer en los sedimentos hidrotermales con grandes gradientes térmicos (hasta 12 °C/m), en cantidades similares a los no-hidrotermales, y a temperaturas entre psicrofílicas y mesofílicas. A profundidades y temperaturas mayores las poblaciones se reducen rápidamente, pero siguen manifestándose hasta temperaturas hipertermofílicas, e incluso son estimuladas por el flujo de agua marina. Según esto, la temperatura por sí sola no limita la presencia de bacterias en sedimentos hidrotermales hasta profundidades de 4 km.

Los experimentos muestran que en presencia de los substratos orgánicos degradables las bacterias con alta actividad de crecimiento pueden moverse a una velocidad mayor que el ritmo de deposición, por lo que no quedan necesariamente atrapadas. Sin embargo, el crecimiento se reduce con la profundidad, de modo que las bacterias ceden ante la velocidad de sedimentación y son enterradas. Las bacterias subsuperficiales pueden utilizar materia orgánica recalcitrante pero de modo muy lento, y parece que han desarrollado una estrategia que engloba alta biomasa con bajo ritmo de crecimiento.

Key words microbial processes · subseafloor sediments · bacteria

Introduction

Between 5 and 10 billion tons of particulate organic matter is constantly sinking in the world's oceans and accumulating as sediment (Jørgensen 1983). The vast majority of this matter is recycled by near-surface microbial activity, but over geological time the remainder accumulates and represents the largest global reservoir of organic carbon (about $15{,}000 \times 10^{18}$ g C; Hedges and Keil 1995), including fossil fuels. Despite this large amount of organic matter in marine sediments, which can be greater than 10 km in depth (Fowler 1990), bacterial processes were thought to be limited to the upper few tens of metres. For example, Morita and Zobell (1955) declare that the base of the marine biosphere occurs at 7.47 m, based on their inability to culture bacteria at this or greater depths. In addition, thermogenic processes, which under certain circumstances lead to oil (100–150 °C) and gas formation (Killops and Killops 1993), are thought to replace any biological processes at about 1 km and around 50 °C. The presence of deep bacterial activity in marine sediments is suggested indirectly by chemical changes in pore water, gas production, modification of organic compounds, presence of bacterial biomarkers and changes in stable isotopes. Until recently, however, the presence and significance of deep bacterial populations had not been confirmed by appropriate microbiological data (Sinclair and Ghiorse 1989). The existence of bacteria in deep formations had been reported; however, these did not involve analysis by various complementary approaches, such as direct microscopy; bacterial activity; viable counts, including demonstration that isolates were adapted for growth under deep sediment conditions; and genetic analysis. Such approaches are required to unambiguously demonstrate the presence and deep origin of these bacteria. In addition, samples had not been analysed over a comprehensive depth range or at multiple sites, which is required to demonstrate that subseafloor bacteria are a consistent and characteristic component of deep marine sediments. In addition, the view was that conditions would become too extreme for life with increasing sediment depth, e.g., decreasing energy sources, increasing temperature and pressure, and decreasing porosity. As a result, reports of bacteria in deep subsurface marine sediments were dismissed as being contaminants from the more active surface layers or possibly due to reactivation of spores or dormant cells.

To resolve this situation, in 1986 an investigation was begun to study bacterial distributions and activity in cores collected by the International Ocean Drilling Program (ODP). ODP is particularly suitable for this research, because they have excellent techniques for collecting undisturbed and uncontaminated core samples, and they routinely obtain geochemical, sedimentological, and other data that are required for interpretation of bacterial distributions. In this study, a complementary suite of microbiological techniques was used, and bacterial distributions were analysed at specific intervals over the complete core depth. Until 1996, 12 sites in the Atlantic and Pacific Oceans and the Mediterranean Sea had been analysed, as shown in *Figure 1*. Total bacterial populations were deter-

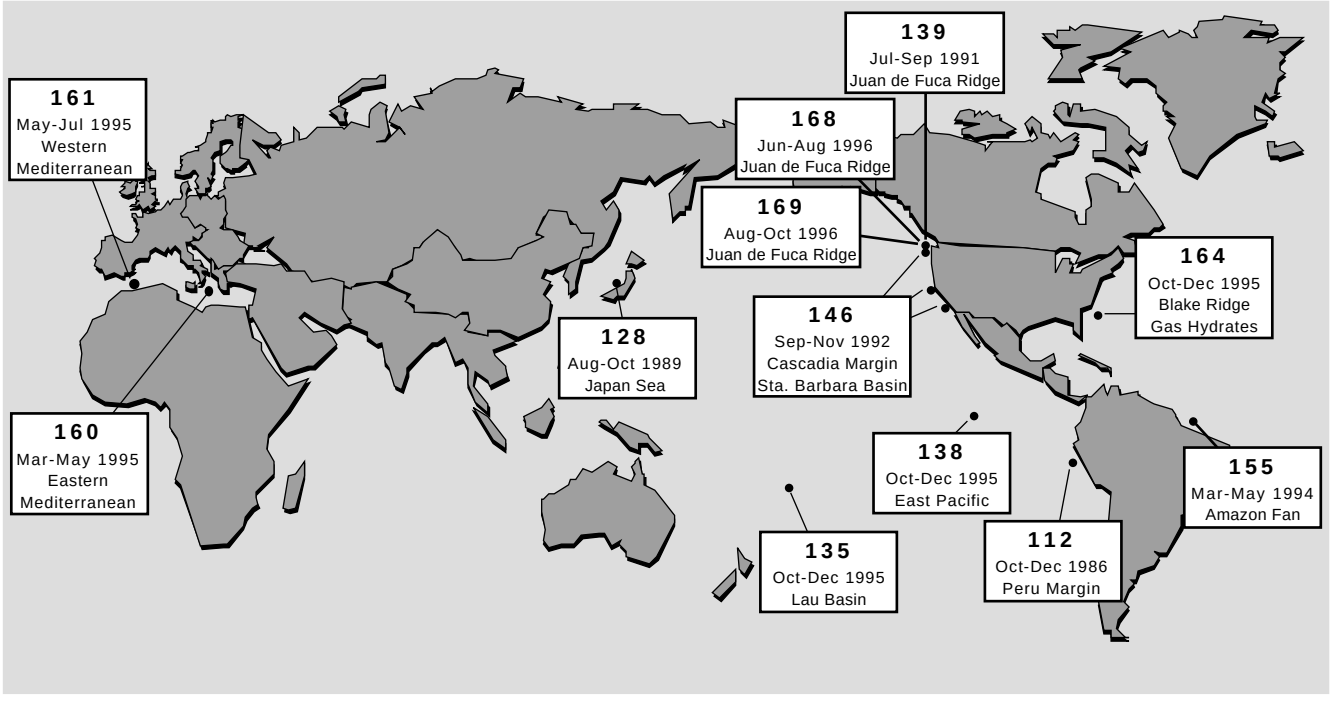

Figure 1 Location of sampling sites for the Ocean Drilling Program (ODP) for subseafloor microbiology during 1986–96. *Numbers* designate individual ODP cruises

Table 1 Maximum rates of potential bacterial activity in subseafloor sediments from four ODP Legs. *nd* No data. Values in *parentheses* refer to data from RV "Darwin" cruise, April–May 1989

Activity	Depth (m)	112 Peru	128 Japan Sea	146 Cascadia	164 Blake Ridge
Sulphate reduction ($nmol\ mL^{-1}\ day^{-1}$)	Top 1	nd (8.74)	24.77	6.85	400.3
	1–10	4.13 (0.17)	1.67	0.021	220.9
	10–100	0.0016 (nd)	0.0018	0.0014	0.88
	Below 100	nd	0.0042	0.0011	1.91
	Below 500	nd	nd	nd	5.89
Methanogenesis (from HCO_3^-) ($nmol\ mL^{-1}\ day^{-1}$)	Top 1	nd (3.69)	0.64	0.0097	0.16
	1–10	0.183 (1.05)	0.48	0.0145	0.13
	10–100	0.046 (nd)	0.09	0.272	0.03
	Below 100	nd	0.03	0.00173	2.73
	Below 500	nd	nd	nd	0.01
Methanogenesis (from acetate) ($nmol\ mL^{-1}\ day^{-1}$)	Top 1	nd	nd	nd	74.01
	1–10	nd	nd	nd	182.3
	10–100	nd	nd	nd	58.3
	Below 100	nd	nd	nd	339.65
	Below 500	nd	nd	nd	1240.2
Methane oxidation ($nmol\ mL^{-1}\ day^{-1}$)	Top 1	nd	nd	0.218	0.085
	1–10	nd	nd	18.58	0.042
	10–100	nd	nd	16.33	173.62
	Below 100	nd	nd	134.53	19.23
	Below 500	nd	nd	nd	56.67
Thymidine incorporation ($fmol\ mL^{-1}\ day^{-1}$)	Top 1	nd (248.7)	476.5	nd	4068.3
	1–10	nd (106.1)	210.9	nd	1206.6
	10–100	nd	0.4	nd	737.1
	Below 100	nd	0	nd	1341.3
	Below 500	nd	nd	nd	204.6

mined at all sites, with more comprehensive microbiological analysis at four locations; activities at the latter are shown in *Table 1*. These sites cover a wide range of oceanographic conditions: water depth from 151 to 3773 m (Leg 112, Peru Margin, and Leg 138, Eastern Equatorial Pacific, respectively); maximum sediment depth from 80.2 to 748.49 mbsf (metres below the seafloor) (Leg 112 and Leg 164, Blake Ridge, respectively); maximum age to 10 my (million years) (Leg 135, Lau Basin); high (Leg 112) to low productivity (Leg 138); high organic matter (6.8% average top 80 m, Leg 112, site 680) to low organic matter (carbonate sediments, Leg 138); semi-enclosed basins and seas (Santa Barbara Basin, Leg 146; Mediterranean Sea, Legs 160 and 161; and Japan Sea, Leg 128); river fan systems (Leg 155, Amazon Fan); accretionary wedge sediments (Leg 146, Cascadia Margin); hydrothermal sediments (Legs 139 and 169, Juan de Fuca Ridge); with sapropels (Leg 160), turbidites (Leg 155), and gas hydrates (Legs 146 and 164).

Methods and techniques used are only outlined in the following sections; however, details are in Parkes et al. (1995) and ODP publications in the references.

Bacterial Populations: Depth and Site Distributions

Total Bacterial Population

Total bacterial populations were determined using the acridine orange direct count (AODC) technique (see Cragg and Parkes 1993) at all sites, resulting in about 890 individual counts. Samples were counted in at least triplicate with a minimum of 200 fields of view counted on each membrane, which provided a significance level of $7 \times 10^4/cm^3$. Where bacterial numbers were very low, however, counts on triplicate membranes were combined, giving a total number of fields of view of >1200.

Bacterial populations were high near the sediment surface (top 1 m between 1.4 and $4 \times 10^9/cm^3$, Japan Sea and Peru Margin, respectively) and decreased rapidly with increasing depth, as shown in *Figure 2*. Although the average populations are still substantial ($2.76 \times 10^6/cm^3$) at the mean oceanic sediment depth of 500 m (Chester 1990), they represent on average a 97% decrease over near-surface populations. This decrease in bacterial populations with increasing depth presumably reflects the preferential utilization of the more readily degradable components of sedimentary organic matter. Thus, not only is organic matter removed during burial, it becomes increasingly recalcitrant.

A summation of bacterial biomass to 500 m equates to a considerable amount of bacterial organic carbon,

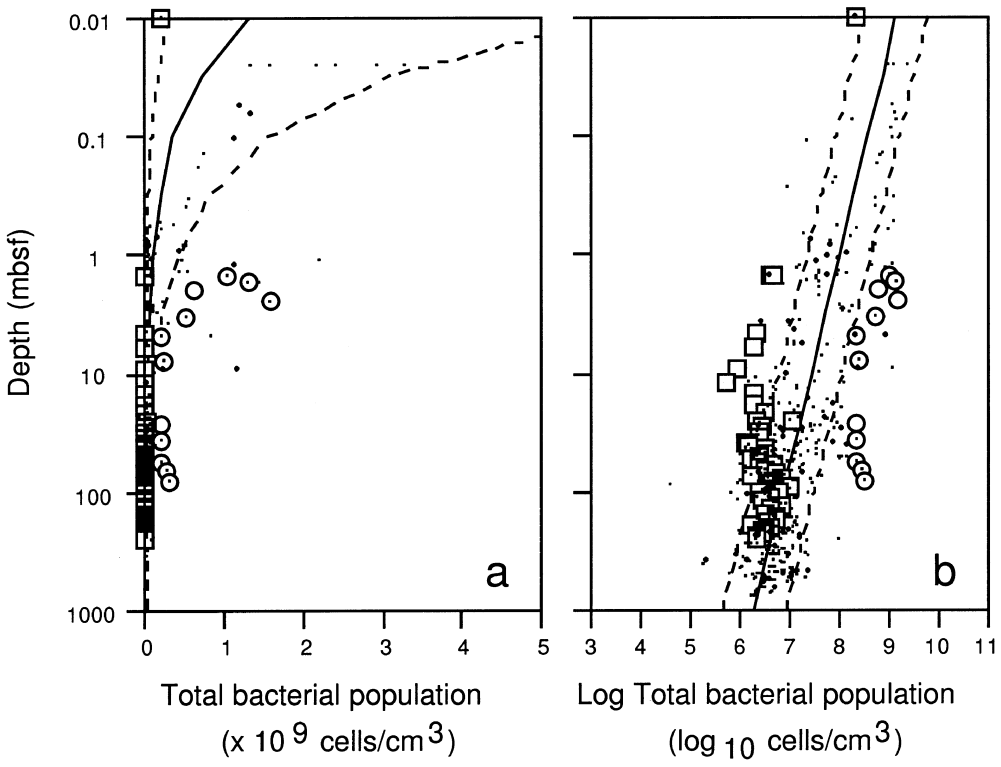

Figure 2a,b Depth distribution of subsurface bacterial populations at all sites shown in *Figure 1*. Bacterial numbers are shown on both **a** linear and **b** logarithmic scales. *Solid curve* represents the general regression-line model, with 95% upper and lower prediction limits shown by *dashed curves* (Cragg et al. 1990, 1992, 1995a, 1995b, 1997, 1998, 1999; Cragg and Parkes 1993; Cragg 1994; Cragg and Kemp 1995; Mather and Parkes in press; Wellsbury et al. in press) (Legs 112, 128, 135, 138, 139, 146, 155, 160, 161, 164, 168, 169). Regression line log Bacterial numbers = 7.98–0.57 log Depth [r^2=0.561]
○ Peru Morgin
□ Eastern Equatorial Pacific

about 1.5 t/ha. Although this amount represents a small percentage of global sedimentary carbon (0.004%), remarkably it is about 10% of the living carbon in the surface biosphere (Parkes et al. 1994). Hence, the discovery of bacterial populations in deep marine sediments has added an additional 10% to the estimate of global biomass.

Bacterial depth distributions in marine sediments vary systematically from the general regression line (*Figure 2*) according to their oceanographic setting. For example, populations are elevated at the shallow-water, high-productivity Peru Margin site (112), whereas at the low-productivity Eastern Equatorial Pacific site (138), bacterial populations are reduced (*Figure 2*). This relationship suggests that the bulk of AODC-positive cells are intact and not dead, because if the majority of cells were dead they must have survived in that state for a considerable length of time to be detected, and hence the consistently high surface-bacterial populations on death would be preserved during burial, obscuring differences in depth distributions between sites. This conclusion is consistent with: (1) the presence of high molecular weight intact DNA, which can be amplified with prokaryotic primers (Rochelle et al. 1994); (2) rapid growth of bacteria in enrichment cultures (Getliff et al. 1992); (3) isolation of bacteria that are physiologically adapted to their deep sediment environment (Bale et al. 1997; Barnes et al. 1998); (4) significant rates of bacterial activity measured by radiotracers (e.g., Cragg et al. 1992); (5) significant correlation ($P<0.001$) between numbers of dividing and divided cells and growth determined by thymidine incorporation (unpublished data); and (6) stimulation of bacterial populations and activity at depth at some sites (e.g., Parkes et al. 1994; Wellsbury et al. 1997). Because bacterial populations do not decrease more rapidly with depth in the deeper layers (*Figure 2b*), bacteria should be present at much greater depths than the deepest samples currently reported (748.49 mbsf, Leg 164, hole 997; Wellsbury et al. in press). Unpublished results from the Woodlark Basin Papua New Guinea, Leg 180, hole 1118, demonstrate a population of about 320,000 bacteria/cm^3 at 842 mbsf. Sediments can be >10 km deep (Fowler 1990). In addition, at several sites bacterial populations increase in deeper zones (e.g., Japan Sea, gas-hydrate deposits, Legs 146 and 164; Cragg et al. 1996; Wellsbury et al. in press, respectively). Thus, deep-sediment bacteria are likely to make an increased contribution to global biomass.

Factors Limiting Bacteria at Depth

Temperature

A factor that could limit bacterial distributions in deeper sediments is the increasing temperature during burial. Bacterial processes are thought to give way to thermogenic processes at about 50 °C and ca 1 km (Killops and Killops 1993). In contrast, bacteria from hydrothermal chimneys exist that can grow at up to 113 °C (Blöchl et al. 1997), but whether these hyper-

thermophilic bacteria are present in subsurface sediments is uncertain. Access to very deep sediments is not yet available to test this directly (113 °C would require about 4 km at a thermal gradient of 30 °C/km). Therefore, sediments within the Juan de Fuca Ridge hydrothermal vent field were investigated (Cragg and Parkes 1993, and unpublished data) because it has large thermal gradients, and hence high temperatures exist at relatively shallow sediment depths.

At the Bent Hill Massive Sulphide site (Leg 169), the temperature gradient is about 0.7 °C/m (Fouquet et al. 1997). Although bacteria are present at all depths, as shown in *Figure 3*, populations below ca. 28 mbsf are significantly lower ($P < 0.0005$) than the general depth trend for non-hydrothermal deep sediments. This deviation increases with depth such that below about 70 mbsf some bacterial populations become non-significant for the first time. The first deviation from the general bacterial depth distribution at 28 mbsf is at a temperature of about 45 °C, the upper temperature limit for mesophilic bacteria. Deeper bacteria would, therefore, have to be thermophiles, which can grow up to about 80 °C. At temperatures greater than 80 °C, a second major decrease occurs in bacterial populations. Bacteria deeper still must be hyperthermophiles, able to grow above 80 °C, and these are present at depths with temperatures very close to the current established maximum temperature for bacterial growth of 113 °C (*Figure 3*).

Throughout the core, geochemical evidence indicates continued bacterial activity (e.g., increases in alkalinity and ammonium, and removal of sulphate; Fouquet et al. 1997). Therefore, bacterial depth distributions at this hydrothermal site cover the full bacterial temperature range from psychrophiles near the sediment surface (2.1 °C; Fouquet et al. 1997) to hyperthermophiles with increasing depth. If a similar situation occurs at greater depth in non-hydrothermal sediments, temperature alone would not be expected to become limiting for bacteria until about 4 km. However, both thermophilic and hyperthermophilic populations are significantly ($P << 0.0005$) lower than those at an equivalent depth in non-hydrothermal sediments. This condition suggests that fewer bacteria can grow at these elevated temperatures and/or that elevated temperatures may compound the limitation of organic matter becoming increasingly more recalcitrant during burial. In addition, this more rapid decrease in bacterial populations in hydrothermal sediments reinforces the assertion that the AODC technique is detecting intact bacterial cells: if buried dead cells were surviving for long periods of time, little or no difference would exist between depth distributions of bacteria in hydrothermal and non-hydrothermal sediments.

However, what is the prospect for the existence of even higher-temperature bacteria and thus for an even deeper and hotter biosphere in marine sediments? Subsurface hydrothermal sediments would seem to be an ideal environment to investigate this question, because the possible presence of very high-temperature bacteria beneath hydrothermal systems has often been speculated (e.g., Deming and Baross 1993). Legs 139 and 169 provided samples from five holes 5–70 m away from active vents with a temperature of about 270 °C. Thermal gradients were all large, ranging from 3–12 °C/m, depending on their proximity to vents. Bacterial populations decreased rapidly with depth, as shown in *Figure 4*. The populations became statistically non-significant between about 80 and 105 °C in a similar stepwise manner with respect to temperature to that previously described for the Bent Hill Massive Sulphide site. However, between about 155 and 180 °C, significant bacterial populations were again present in all holes (4) that reached this temperature. Although the presence of bacteria at such high temperatures is surprising, and only few data exist, the

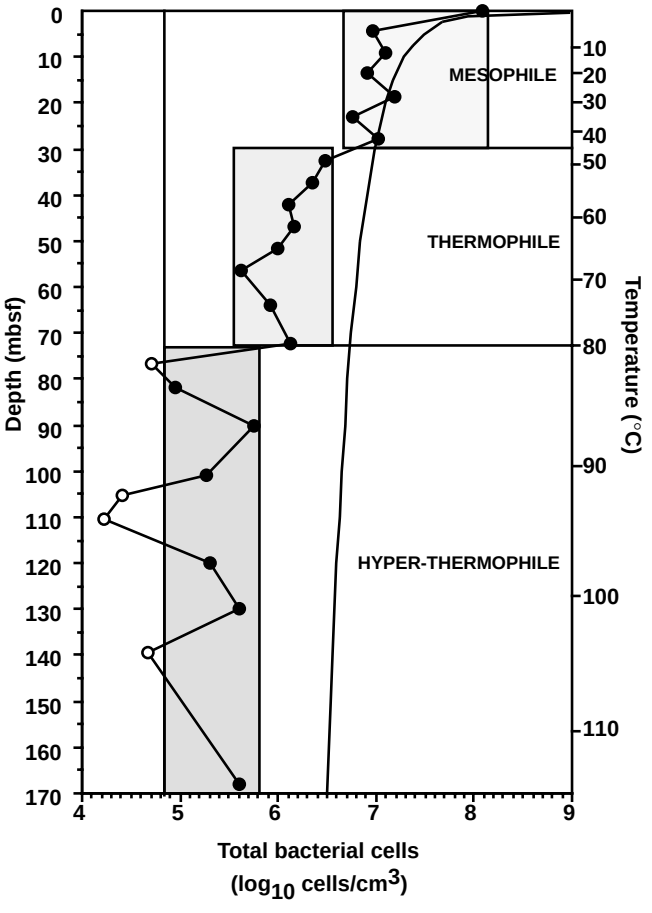

Figure 3 Bacterial distributions in the Juan de Fuca hydrothermal vent field (Leg 169), Bent Hill Massive Sulphide site, with respect to depth and temperature. *Solid curve* represents the general regression-line model (*Figure 2*). *Solid vertical line* is a significance limit (7×10^4/cm^3); counts below this limit are shown as *hollow circles* and are not significant. *Shaded areas* denote those bacterial populations living within sediments at typical temperature ranges for meso- thermo- and hyperthermophilic bacteria

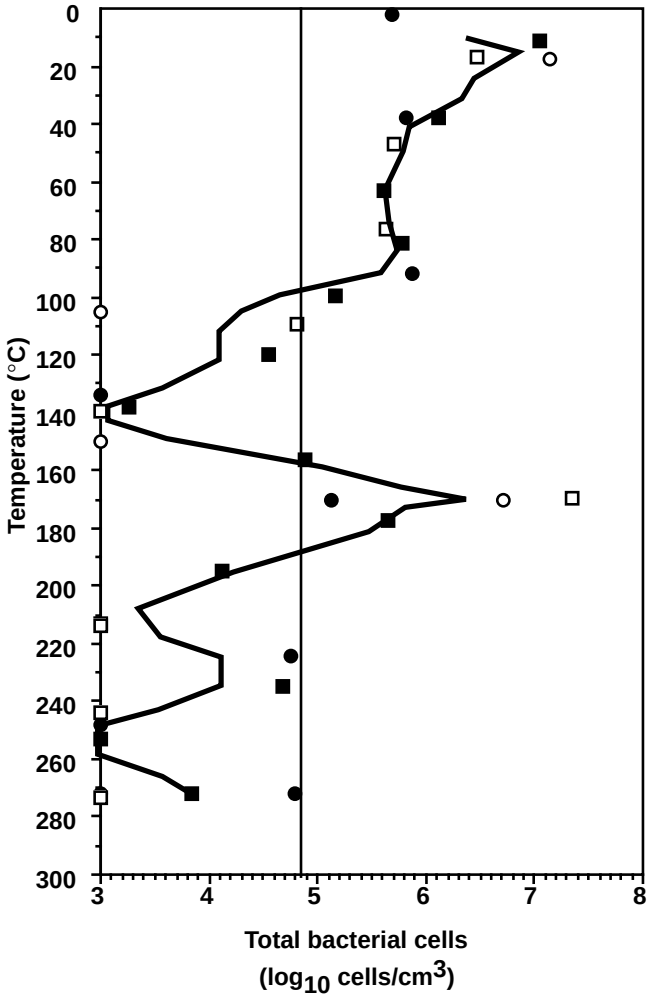

Figure 4 Distribution of subsurface bacterial populations with respect to temperature at sites between 5 and 70 m away from active 270 °C vents in the Juan de Fuca hydrothermal vent field, Leg 139, holes 858B (*solid squares*) and 858D (*open squares*); and Leg 169, holes 1036A (*solid circles*) and 1036B (*open circles*). *Heavy line* represents the moving average calculated from all data. *Solid vertical line* is a significance limit (7×10^4/cm^3); counts below this limit are not significant

consistent presence of bacteria at great depth in all four high-temperature holes obtained in the same area on two separate ODP Legs strongly suggests that bacteria present at high temperatures are a consistent and characteristic feature of subsurface hydrothermal systems.

The presence of intact bacteria at these high temperatures does not necessarily mean that they are growing at these temperatures. Hydrothermal sediments are active hydrological systems where hydrothermal fluid is actively venting. In addition, seawater recharge, subsurface mixing of these fluids, and lateral fluid flow are occurring (Stein et al. 1998). At all four sites, evidence exists for subsurface seawater flow (Davis et al. 1992; Fouquet et al. 1997). Elevated numbers of high-temperature bacteria appear to be associated with seawater mixing in the subsurface with higher chloride and higher temperature hydrothermal fluid. Possibly, a portion of bacteria directly in seawater or bacteria entrained from shallower sediments during seawater transport survive high-temperature lysis (Membrillohernandez et al. 1995) long enough to appear as very high-temperature subsurface bacteria. Conversely, these bacteria may occupy a unique very high-temperature subsurface habitat resulting from the mixing of recharging seawater and hydrothermal fluid. More research is required to resolve this issue, but these results do demonstrate the presence of a significant subsurface bacterial population in a hydrothermal system and at very high temperatures.

Age and Porosity

If temperature does not become limiting to bacteria until several kilometres depth in non-hydrothermal sediments, do other factors limit bacteria before these depths are reached? Bacterial distributions in non-hydrothermal sites are highly significantly correlated with depth, as shown in *Figure 5a* ($r^2 = 0.559$, $n = 822$), but which of the various factors changing with depth, such as age and porosity, control bacterial distributions? Plotting bacterial numbers against either age or porosity also produces a significant correlation, but neither relationship is as good as with depth (r^2 for depth = ca. $\times 2$ r^2 for age and ca. $\times 3$ r^2 for porosity). Sediment age should, in general, correspond to increasing recalcitrance of organic matter during burial and thus could be considered to have a major, if not the major, control on bacterial depth distributions; however, this seems not to be the case, as indicated in *Figure 5b*. Furthermore, as shown in *Figure 5c*, porosity appears to be even less important than age, possibly because bacteria at even 750 mbsf only occupy a very small percentage of the total pore space (about 0.0002%), and hence bacteria are not limited by the space available.

That any degradable organic matter remains after 10 mya (*Figure 5b*) is remarkable, unless conditions are changing with depth to improve the degradability of buried organic matter. Such a process could be reflected in the strong relationship between bacterial numbers and depth at the variety of ODP sites studied (*Figure 5a*) and could also explain the presence of bacteria in ancient sediments. Temperature is a factor that consistently increases with depth, independent of age and porosity, and at high temperatures (> 80 °C; Lewan and Fisher 1994) compounds with low molecular weights are formed by thermogenic modification of organic matter. Under some circumstances, this process results in the formation of fossil fuels (above about 100 °C). Hyperthermophiles can grow in excess of 100 °C and, therefore, might be stimulated by the formation of compounds with low molecular weights and the enhanced reactivity of organic matter at high temperatures. This hypothesis is supported by

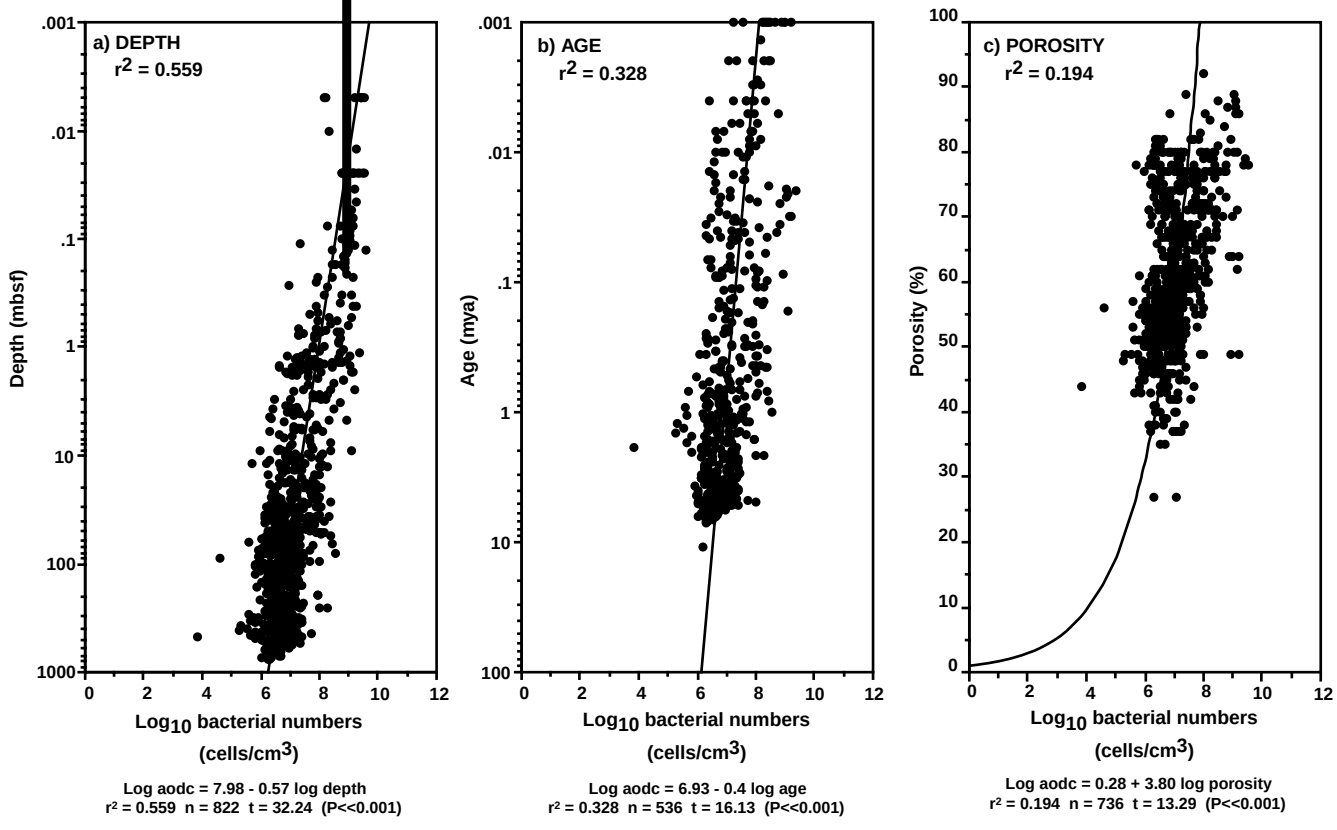

Figure 5 Correlation of non-hydrothermal subseafloor bacterial populations with **a** depth, **b** age, and **c** porosity

increased concentrations of volatile fatty acids with low molecular weights and stimulation of bacterial activity in deep sediments containing gas hydrates. The process is also demonstrated by results of laboratory experiments that model heating during sediment burial (0–100 °C; Wellsbury et al. 1997).

Biodiversity

Various physiological types of bacteria have been enriched and quantified [most probable numbers (MPNs); Parkes et al. 1995] from deep sediments. These types include aerobic ammonifiers; nitrate-reducers, both to ammonia and nitrogen; fermentative anaerobic heterotrophs; sulphate reducers, including acetate, lactate, and methane utilizers that produced CO_2 from CH_4 in initial enrichments but for which activity was not sustained on subculture; methanogens; acetogens; and anaerobic hexadecane oxidizers. Population sizes ranged from zero to $10^5/cm^3$ and generally decreased with increasing depth. With the exception of Blake Ridge, culturable bacteria represent a very small proportion of the direct count (about 0.00001–0.6%). Despite all the recognized limitations of the viable count technique, it can provide useful information, and this is reinforced in these deep sediments. The distribution of viable populations often correlates with measurements of corresponding bacterial activity and/or geochemical changes. For example, viable sulphate-reducing bacteria correlated with sulphate reduction rates and pore-water sulphate, $P < 0.002$; and viable methanogens correlate with rates of methanogenesis, $P < 0.05$. The viable populations, especially the anaerobic heterotrophs, in Blake Ridge gas-hydrate sites are larger than at the other sites, which presumably reflects intense biogeochemical activity (Wellsbury et al. in press) at Blake Ridge.

Pure cultures of sulphate-reducing bacteria were isolated from both the Japan Sea (Parkes et al. 1995) and the Cascadia Margin (Barnes et al. 1998). These bacteria were barophiles, and their pressure optimum for sulphide production corresponded to the calculated pressure at the depths from which they were isolated. Two isolates from the Japan Sea, 80 and 500 mbsf, were fully characterized and these are a new species, *Desulfovibrio profundus* (Bale et al. 1997). The barophilic activity of these sulphate-reducing bacteria confirms their deep-sediment origin and also demonstrates that deep-sediment bacteria are well adapted to their environment.

Undegraded bacterial DNA with high molecular weight has been directly isolated from deep sediments from the Japan Sea (Rochelle et al. 1992) and the Cascadia Margin (unpublished results), which confirms the presence of active bacteria to 503 mbsf (deepest

sample). Polymerase chain reaction (PCR) amplification of DNA extracted from the Japan Sea and sequencing of the 16S rRNA gene demonstrate the presence of bacteria belonging predominantly to the α, γ, and δ proteobacteria (including sequences related to sulphate-reducing bacteria), plus some gram-positive bacteria (Rochelle et al. 1994). In addition, a novel JAP504 cluster of sequences was identified that was not related to any cultured bacteria. Various (eu)bacterial and archaeal 16S rRNA gene sequences have also been obtained from the Cascadia Margin gas-hydrate site; these include methanogens to 234 mbsf (deepest sample; unpublished results).

Types, Rates, and Distribution of Bacterial Activity

Methodological Considerations

In the laboratory, rates of bacterial activity were determined in intact minicores that were injected with radiotracers and incubated under anaerobic conditions for three different time periods, in triplicate, at the mean downhole temperature and at 1 atm (Parkes et al. 1995). All handling was conducted under aseptic and anaerobic conditions. Immediately after injection, one minicore was processed as a time-zero control. Activity in this minicore was stopped by freezing or autoclaving. Incubation times increased with depth and varied with isotope. For example, for Leg 164 these were thymidine incorporation 30 min to 36 h; acetate turnover 1 h to 2 weeks; sulphate reduction, methanogenesis, and methane oxidation 18 h to 48 days (Wellsbury et al. in press).

Although activity was measured in intact minicores, rates obtained are considered as potential and may differ from in situ rates. Reasons for the possible discrepancy include: (1) the sediments were depressurized on coring; (2) due to a ban on the use of radioactive isotopes on board the drill ship, samples had to be stored and transported (4 °C and anaerobically) before analysis a few weeks later; (3) incubation could not be conducted under in situ conditions, because these consistently varied as a function of sample depth; hence, as a compromise, a single set of standard incubation conditions was used, namely at mean down-hole temperature and at 1 atm.

Despite these problems, the rates obtained are believed to reliably reflect bacterial processes in situ. Various lines of evidence support this conclusion:

1. Radiotracer activities correlate with corresponding geochemical changes. For example, sulphate reduction rates correlate with pore-water sulphate and total sulphide (Cragg et al. 1992).
2. Radiotracer activities correspond to stable isotopic values and changes that are distinctive for bacterial activity. For example, anaerobic methane oxidation produces isotopically light carbonate, and sulphate reduction produces isotopically light sulphides (Parkes et al. 1996).
3. The activities also correlate with corresponding mineralogical changes (Musgrave et al. 1997; unpublished results).
4. The activities correspond to rates of sulphate removal during long-term anaerobic incubation (6.5 years) of an intact WRC from the Peru Margin at 4 °C. Long-term sulphate removal was 4.9 nmol cm^{-3} day^{-1}, which agrees closely with previously measured radiotracer rates of 2.0 nmol cm^{-3} day^{-1} for depths 1.2–2.0 mbsf (Parkes et al. 1995).

The reliability of bacterial activity measurements conducted on stored cores may seem surprising, especially to those working on near-surface sediments where even short-term storage can considerably modify measured rates. However, effects of storage for a few weeks of these deep sediments that have been buried over geological time scales is probably insignificant. In addition, because bacterial populations must be severely energy limited, restricted potential exists for changes in rates. This effect has been demonstrated for other subsurface environments; for example, Madsen and Bollag (1989) observed that anaerobic storage of subsurface sediments from the Savannah River Plant, South Carolina, USA, for as long as 4 months had negligible effect on microbiological activity. The deepest sample was from 262 m. Some disturbance of the sample during collection is inevitable; for example, depressurisation always occurs. However, any effect on rates may be both restricted and temporary, due to energy limitation. In this study, activity measurements obtained for three different incubation periods are generally consistent, despite the inherent variability of using minicores, which are not true replicates. In addition, rates tend to become more linear in deeper samples, even though incubation periods are longer. These results indicate that bacterial activity is relatively consistent over time and thus long incubations and storage have limited effect on measured rates, particularly in deeper samples.

Rates of Bacterial Activity in Deep Subsurface Marine Sediments Compared to Rates in Other Environments

Isotopes have been used to measure rates of nitrate turnover to N_2 and NH_4^+, sulphate reduction, acetate oxidation, methanogenesis from both H_2/CO_2 and acetate, methane oxidation, and thymidine incorporation into DNA (bacterial growth) in subseafloor sediments. It is difficult, however, to directly compare rates of bacterial activity measured by the intact minicore approach, which allows rates to be measured in the original sediment matrix, with rates in other subsurface environments. In these environments, rates are usually measured in manipulated samples ranging from (1) rock or sediment slurries (e.g., Fredrickson et al. 1997), sometimes amended with bacterial substrates (e.g., Jones et al. 1989), including hydrogen (e.g., Cha-

pelle and Bradley 1996); to (2) mixtures of groundwater and mineral salts, sometimes with the addition of hydrogen gas (e.g., Kotelnikova and Pedersen 1998), which approach conditions of bacterial enrichment cultures. Even just slurrying can dramatically alter rates, especially where bacteria are heterogeneously distributed and limited by supply of either electron acceptors or donors (Krumholz et al. 1997). Also, rate measurements with the addition of substrates can only demonstrate the potential presence of a particular bacterial process and not whether, or at what rate, this is expressed under in situ conditions. Despite these problems, however, results from these various approaches indicate that bacteria are present and active in other deep subsurface environments and able to utilize ancient organic matter over geological time scales (since Late Cretaceous) and great depths (2.8 km; Fredrickson and Onstott 1996). Included are ancient marine sediments that have subsequently been uplifted and are now on land (Chapelle and Bradley 1996; Fredrickson et al. 1997; Krumholz et al. 1997). These results are consistent with and reinforce results from deep subseafloor sediments.

Comparison of subseafloor bacterial activities can, however, be made with intact core radiotracer measurements made in other environments. With the exception of rates in the near surface of Blake Ridge, measured maximum rates of sulphate reduction (*Table 1*) are at or well below the lower range for near-surface coastal sediments (20–150 nmol cm^{-3} day^{-1}; Cragg et al. 1992; Ferdelman et al. 1997). Sulphate-reduction rates in near-surface Blake Ridge sediments are unexpectedly higher than at the Peru Margin upwelling, a high-productivity site, but they are still within and at the lower end of the ranges for environments with high organic carbon contents. An example is the shallow (45 m) Chilean, upwelling coastal sediments, with rates of 170–4670 nmol cm^{-3} day^{-1} (Ferdelman et al. 1997). These high rates of sulphate reduction at Blake Ridge are still surprising, because this is a relatively deep-water site (about 2800 m); however, the high rates may reflect the intense biogeochemical activity at gas-hydrate sites, which ultimately may influence hydrate formation (Wellsbury et al. in press). Rates of sulphate reduction at deeper depths are low (*Table 1*), because sulphate is removed, but they can become stimulated by the renewed presence of sulphate at depth. This sulphate may result either from a brine incursion from below (Peru Margin and Eastern Flank of the Juan De Fuca Ridge; Parkes et al. 1990; Mather and Parkes in press), or from anaerobic sulphide oxidation (Cascadia Margin; Cragg et al. 1996). In addition, deep sulphate reduction can be stimulated by thermogenic methane from below, providing a new deep energy source, as shown in *Figure 6* (Parkes et al. 1994, and later discussion).

In sediments from the Peru Margin and Japan Sea, rates of H_2/CO_2 methanogenesis, as measured by reduction of $H^{14}CO_3^-$ to $^{14}CH_4$, are highest in the near-subsurface (about 1 m) and tend to peak just below the maximum in sulphate-reduction rates (e.g., *Figure 6*). This change in the dominant anaerobic terminal oxidation reaction occurs at surprisingly high sulphate concentrations, i.e., 16–17 mM. In marine sediments, sulphate-reducing bacteria are thought to be able to out-compete methanogens for common substrates at sulphate concentrations down to about 3 mM (Capone and Kiene 1988). Hence, factors other than sulphate concentration may be controlling the competition between sulphate reducers and methanogens at these sites (Parkes et al. 1990). Near-surface rates of H_2/CO_2 methanogenesis are consistent with rates in coastal sediments with oxic water columns (<1 nmol cm^{-3} day^{-1}; e.g., Kuivila et al. 1990), except for the high-productivity Peru Margin site, which has higher rates (*Table 1*). At deeper depths in the Peru Margin and Japan Sea, rates of H_2/CO_2 methanogenesis, although much lower than in the near-surface, do not decrease as much as rates of sulphate reduction; the decrease from 1 m to 10–100 m for sulphate reduction is about 100 times greater than for H_2/CO_2 methanogenesis (*Table 1*). This difference is because methanogenesis does not become electron-acceptor-limited (CO_2), unlike sulphate reduction. At the two gas-hydrate sites, Cascadia and Blake Ridge, in contrast to the other sites, deep rates of H_2/CO_2 methanogenesis are actually greater than those at the near-surface, and this elevated activity is focused around the gas-hydrate zone (Cragg et al. 1995a, 1995b; Wellsbury et al. 1997). Acetate is another important substrate for methanogenesis, and rates of acetate methanogenesis are also stimulated within and below the hydrate zone at Blake Ridge. Very high rates are obtained, about 10^2 higher than near-surface rates (Wellsbury et al. 1997).

Methane, in addition to being a bacterial product, is a potential energy source for other bacteria, even under anaerobic conditions. Although no organism that can anaerobically consume methane has been isolated, evidence from laboratory studies (Zehnder and Brock 1979; Hoehler et al. 1994; Harder 1997) and direct measurement using ^{14}C-CH_4 (Kosiur and Warford 1979; Iversen and Blackburn 1981; Iversen and Jørgensen 1985; Ward et al. 1987) indicates that the process occurs. In addition, geochemical data demonstrate that the process is environmentally significant (Suess and Whiticar 1989; Masuzawa et al. 1992; Schulz et al. 1994). Although sulphate-reducing bacteria have often been implicated with anaerobic methane oxidation, recent evidence from a methane seep site suggests a novel group of Archaea, peripherally related to methylotrophic methanogens, may also be involved (Hinrichs et al. 1999). Anaerobic methane oxidation also occurs in deep sediments. Using the ^{14}C-CH_4 tracer technique, methane oxidation was shown to occur throughout the two gas-hydrate sites (*Table 1*) and indirect evidence suggests that methane oxidation occurs in deep sediments from the Japan

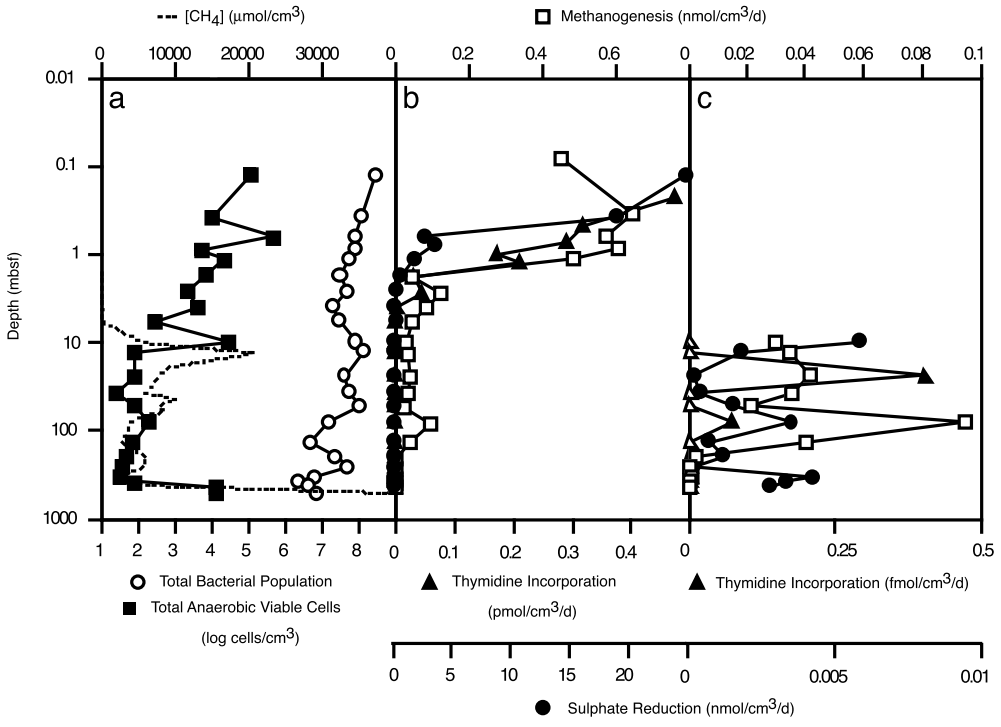

Figure 6 Depth changes in radiotracer measurements of potential bacterial activities and comparison with bacterial populations and methane gas concentrations in sediments from the Japan Sea, ODP Leg 128. **a** Distribution of total bacterial populations (*open circles*), total anaerobic viable count (*solid squares*) and methane concentration (*dashed line*) to 517.9 mbsf. **b** Bacterial activity and viable populations: sulphate-reduction rate (*solid circles*), methanogenesis (*open squares*), thymidine incorporation rate (*solid triangles*). **c** Section from 9.6 and 517.9 mbsf with expanded scale for clarity. *Open symbols* denote zero-value data

Sea (Parkes et al. 1994); methane oxidation was not measured in the Peru Margin. Near-surface rates are similar to the range for shallow marine sediments (0.002–0.1 nmol cm^{-3} day^{-1}; Iversen and Jørgensen 1985). However, rates of methane oxidation increased dramatically with depth, to more than 10^3 greater than near-surface rates. This presumably reflects the large amount of methane in, below, and fluxing through gas-hydrate deposits (Dickens et al. 1996), and it demonstrates that methane is an important deep energy source in sediments containing gas hydrate. Gas hydrates may reflect an extreme example of deep methane utilization, which may also occur in sediments without gas hydrates but at much reduced rates (see below).

Depth Distribution and Comparison of Rates with Other Measurements of Bacterial Activity

Detailed measurements of bacterial rates were conducted at four ODP sites (*Table 1*). At all sites, overall bacterial activity is high near the sediment surface (about the upper 5 m), and decreases rapidly with depth, reflecting changes in bacterial populations (*Figures 2* and *6*). In addition, the type of bacterial activity changes with depth, usually as a response to geochemical changes, some of which themselves are bacterially mediated; for example, sulphate removal stimulates methanogenesis. The deepest analysed samples are from 690.6 mbsf at site 995 on the Blake Ridge, Atlantic Ocean (Leg 164), where measurable rates were observed of sulphate reduction, H_2/CO_2 methanogenesis, acetate oxidation and methanogenesis, and methane oxidation (Wellsbury et al. in press; note, nitrate turnover was not measured).

Results from the Japan Sea (Leg 128; Cragg et al. 1992) are described below as an example of the depth distributions of radiotracer bacterial activities and their relationship to other indices of bacterial activity (*Figure 6*). Sulphate reduction is greatest in the near-surface and is quantitatively the most important anaerobic process. Sulphate reduction is significantly correlated with pore-water sulphate ($P < 0.002$); below 9.62 mbsf, sulphate is reduced to low concentrations (< 0.95 mM). Sulphate removal limited sulphate reduction and enabled methanogenesis to peak just below the maximum in sulphate-reduction rates (see Capone and Kiene 1988). Below this near-surface active zone to 503 mbsf (deepest sample), rates of sulphate reduction and methanogenesis are low but significant, as demonstrated by increases in total sulphide (data not shown) and methane in deeper layers (*Figure 6*). Not surprisingly, because sulphate concentrations are low in the deeper sediments (maximum 0.92 mM), methanogenesis is quantitatively more important than sulphate reduction. Rates of methanogenesis vary in the deep zone, as reflected in variations in concentrations of methane gas (*Figure 6*). Between 12.6 and 503 mbsf, a significant positive relationship ($P < 0.05$) exists between total numbers of viable anaerobic bacteria and methane concentrations. This relationship suggests that a subset of the bacterial population might be able to use methane as a deep energy source. This hypothesis is reinforced by the significant increase in viable anaerobic bacteria below about 360 mbsf, which corresponds with a large

increase in methane concentrations (*Figure 6*). This deep methane, however, is of thermogenic origin (based on C_1 to C_2 ratios; Ingle et al. 1990), which suggests that deep-sediment bacteria might be able to utilize geothermally derived energy sources and, therefore, not be limited to the metabolism of buried organic matter (Parkes et al. 1994). This ability may not only enable bacterial populations to increase in deep sediments but also enable them to be present much deeper than has previously been thought possible based on the assumption that bacterial energy sources continuously decrease with depth.

Sulphate-reducing bacteria have been implicated in anaerobic methane oxidation (e.g., Iversen and Jørgensen 1985), and a small but sustained increase in sulphate reduction rates coincides with the increase in anaerobic bacteria and methane gas (*Figure 6*). In addition, sulphate-reducing bacteria were subsequently isolated from these deep sediments (Bale et al. 1997). These data provide circumstantial evidence that methane stimulates deep sulphate reduction.

Bacterial Growth and Motility

The mobility of bacteria within sediments or rocks determines whether they are trapped within the originally deposited sediment layer or able to move to exploit more recent sediments or new energy sources, such as deep-sourced thermogenic gases and other compounds. If entombed within the deposited sediment, deep bacteria should be lineal descendants of the bacteria originally present in the surface sediment and thus, intriguingly, this community would have survived over millions of years within the same sediment layer. Movement may be achieved through motility, growth, diffusion, or a combination of these processes. In the terrestrial subsurface, particularly in the unsaturated zone, surface bacteria can potentially be introduced by groundwater flow. In contrast, marine sediments are saturated with pore water, which is slowly expelled during compaction, thus limiting the potential for fluid flow from the surface and, hence, introduction of surface bacteria. Seawater flow into the marine subsurface, however, can occur in certain circumstances, such as in active hydrothermal systems, where discharge of high-temperature hydrothermal fluid produces a hydrologically active system, with seawater recharge to considerable depths (Karl 1995). This process can have a major impact on pore-water chemistry and is usually easily recognized (Cragg and Parkes 1993).

The following studies were conducted to determine the potential for bacterial growth and motility in deep marine sediments.

Experimental Determination of Movement of Indigenous Bacteria in Intact Deep Sediments

Four vertical minicores from an intact core from the Peru Margin (ODP Leg 112, 2.2–2.3 mbsf; Cragg et al. 1990) were taken and sterilized by gamma irradiation (about 11.5 K Gy, Isotron plc, Swindon, UK), before being replaced into the original sediment core. The core had been stored at 4 °C under anaerobic conditions since collection; storage period was 5.7 years (Cragg et al. 1992). The core was slightly compressed to ensure intimate contact between the irradiated minicores and the original sediment and incubated anaerobically at 25 °C. A minicore was removed at 34, 197, and 478 days and a central slice, 2 cm by 2 mm across the diameter, was obtained for the full length of the minicore. This central slice was further divided into 10×2-mm sections, thereby providing samples from one edge of the sterile sediment plug to the other for determination of total bacterial numbers (AODC). At each time interval, samples were also taken from the non-irradiated core.

Irradiation destroyed over 99% of intact cells and all culturable cells (anaerobic heterotrophs; Cragg et al. 1992; data not shown). During incubation, bacteria moved from the original sediment into the irradiated sediment plug, as shown in *Figure 7*. By 478 days the bacterial population in the middle of the irradiated sediment plug had reached 21% of the original bacterial population in the non-irradiated section of the core (based on day 34 bacterial counts), and the bacterial population at the edge of the plug had increased by 226%. This presumably demonstrates effective utilization of bacterial necromass (Parkes et al. 1993) and cell lysis products, produced during irradiation, by bacteria from the non-irradiated sediment. Estimate of the conversion efficiency of the killed cells into new bacterial cells within the irradiated plug was surprisingly high at about 42%. However, the situation was complicated, because bacteria also increased outside the plug by a factor of about 1.5 (*Figure 7*). This increase probably reflects diffusion of cell-lysis products from the irradiated plug into the surrounding sediment. This increase is possibly also an effect of increased temperature (25 °C), which enhances the degradability of organic matter within the sediment (see previous discussion and Wellsbury et al. 1997). Thus, the increase in bacteria within the irradiated sediment plug has been adjusted for stimulation of bacteria in the non-irradiated section. Despite this complication, bacterial cell debris or necromass greatly stimulates bacterial growth rates. These rates are about 20 times higher within the irradiated plug than outside it, and presumably a similar situation occurs in sediments in situ. This process might be particularly important, for example, when seawater is drawn through hydrothermal sediments, because bacteria will lyse in the high-temperature subsurface, providing substrates for high-temperature bacteria (*Figure 4*).

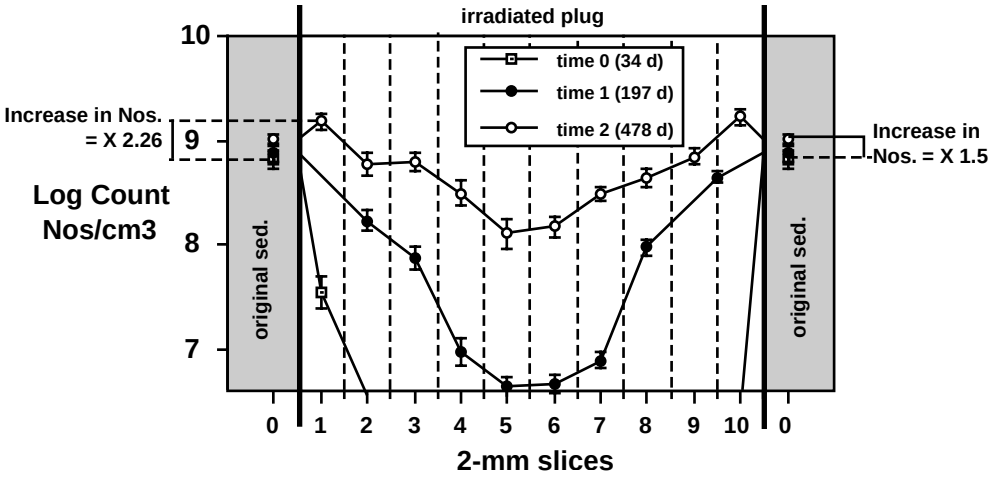

Figure 7 Movement of indigenous bacteria in intact subseafloor sediments (Peru Margin sediments, Leg 112) during incubation at 25 °C. Irradiated subcores were replaced into the original intact Whole Round Core for 34, 197, and 478 days prior to analysis of total bacterial populations in 2-mm sections

The rate of bacterial movement into the irradiated sediment was about 18 mm/year. This is considered to be near maximum, due to the ready supply of organic substrate and high incubation temperature. Estimates based on culturable anaerobic heterotrophs were statistically identical, and the data are not shown. These rates of bacterial movement, however, are greater than two orders of magnitude faster than high marine-sedimentation rates (about 1–3 cm 10^{-3} $year^{-1}$). Hence, under favourable conditions, bacteria would be able to move into depositing detrital material and thus exploit fresh organic matter and not necessarily be trapped within it. Bacterial movement should be closely related to activity and growth, and growth rates in this experiment were similar to those obtained from [^{3}H]-thymidine incorporation measurements in other near-surface ODP cores ($10^5 - 10^6$ cells mL^{-1} day^{-1}).

These findings show that under natural conditions, near-surface sediment bacteria should be sufficiently motile to avoid burial. However, except for the biogeochemically active Blake Ridge gas-hydrate site (Leg 164), growth rates decrease rapidly below the upper 10 m (10^2 cells mL^{-1} day^{-1}, or below detection; *Table 1*). Even slower growth rates (0.15 cells mL^{-1} day^{-1}) were measured in intact ODP cores (Leg 112, Peru Margin, hole 679C, 1.6, 19.7, and 74.8 mbsf) incubated at 25 °C for up to 525 days. Therefore, bacterial movement in deeper layers would be considerably slower than measured in this experiment, and hence bacteria would not be able to keep up with sedimentation rate and become buried, unless additional energy sources become available.

Bacterial Populations in Mediterranean Sapropels

An example of bacteria being buried in specific horizons is provided by bacterial populations within sapropels in Mediterranean sediments (ODP Leg 160; Cragg et al. 1998). Sapropels are discrete layers of elevated, buried organic matter that are potential energy sources for bacteria over millions of years. At a site on the Mediterranean Ridge (hole 969B; Cragg et al. 1998), four sapropels were sampled as part of a depth sequence of bacterial distributions to 97.8 mbsf; the profile is shown in *Figure 8*. Bacterial populations were high at the near-surface (9.79×10^8) and decreased rapidly by >99.8% at 11.9 mbsf. Thereafter, except in the sapropels, bacterial populations remained relatively constant. In the four sapropels, a significant ($P < 0.05$) and reproducible elevation occurred in bacterial populations. Replicate counts at 19.9 and 46.9 mbsf were not significantly different. Not only did sapropels contain high organic matter (many over 10% and a maximum of 30.5%) but also sulphate supplied by deep evaporitic deposits resulted in sulphate concentrations increasing with depth, from 31.2 mM in overlying water to 39.7 mM in the deepest section, 102 mbsf. Thus, sulphate was consistently present in non-limiting concentrations for sulphate reduction (>3 mM; Capone and Kiene 1988).

At depths where organic carbon concentrations are closer to those of normal marine sediments (about 0–3%), a significant correlation exists between organic carbon and bacterial populations ($P < 0.05$). However, when depths with higher organic carbon are included, this relationship breaks down, suggesting that high concentrations of organic carbon, such as in the sapropels, are effectively present in excess and are not limiting for bacteria. Thus, bacteria within the sapropels should have non-limiting conditions for growth, and the reason that the layers with high organic-carbon contents remain is that the organic carbon is recalcitrant and only enables very slow bacterial growth. Under these conditions, bacterial populations would be expected to increase in sapropels during burial. Thus, deeper and older sapropels should have higher bacterial populations compared to adjacent non-sapropel sediments than do younger and shallower ones. This situation is exactly what occurs (*Figure 8*), even though the deepest sapropel is 4.7 my. Plotting the elevation in bacterial populations against sapropel age

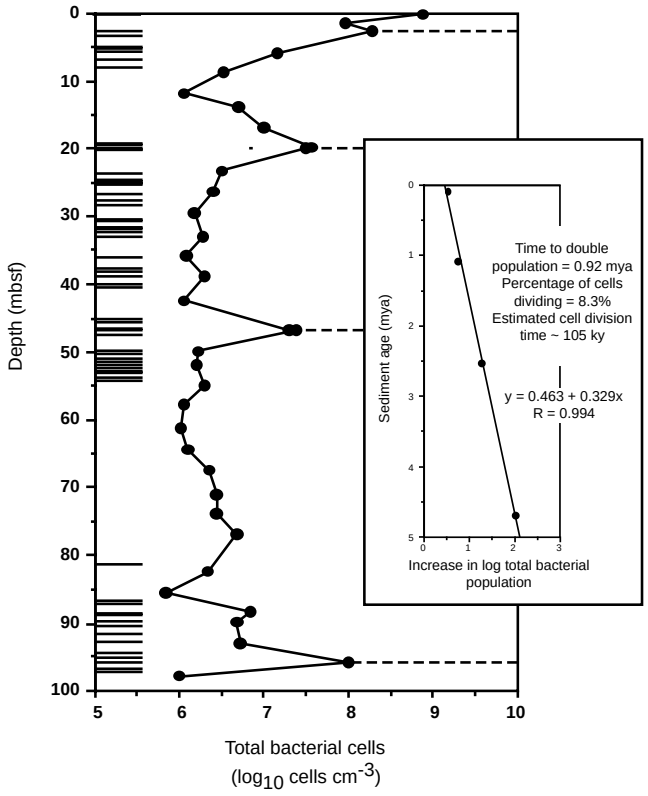

Figure 8 Depth distributions of bacteria in sediments containing sapropels from the Mediterranean Sea, Leg 160. Sapropel layers with high organic matter are shown as *horizontal dark bands on left-hand axis*. Significant increases in bacterial population numbers occurred in all four sapropels analysed (shown by *horizontal dashed lines*). Relative population increases within these sapropels are plotted against sediment age (*inset*), to provide an estimate of bacterial division time

gives a significant linear relationship (*Figure 8*, inset), the slope of which is growth rate, which gives a bacterial doubling time of 0.92 mya. The percentage of dividing and divided cells is 8.3%; thus, the average division time for the active population is a remarkable ~105 ky.

This very long division time is a maximum estimate, because it does not include cell death. Although the number of dividing and divided cells is an index of growing cells (Getliff et al. 1992), the actual number of actively growing bacteria is likely to be overestimated by the approach when used in sediments (Newell and Fallon 1982). These data, however, demonstrate the ability of bacteria to grow on old and deeply buried organic matter, although this material is recalcitrant and hence only enables very slow bacterial growth. This scenario also explains how organic matter can persist in deep sediments even in the presence of significant bacterial populations, because the organic matter can only be degraded extremely slowly. Similar recalcitrant organic matter should be present in non-sapropel sediments, but much reduced and at concentrations probably limiting to the bacteria. Hence, bacterial populations are much lower, and degradable organic matter decreases with depth, resulting in a decline in bacterial populations during burial (*Figure 2*). In deep non-sapropel sediments, any energy obtained by bacteria from buried organic matter may be used for cellular maintenance and thus survival rather than growth. This condition enables surprisingly high biomasses to be maintained, as reflected in long division times. How they accomplish this is a major challenge for future research. If conditions change at depth, such as the occurrence of brine incursions, lateral fluid flow, or temperature increases, more of the organic matter becomes available, thereby enabling an increase in bacterial activity.

Discussion and Conclusions

The great depth and age of marine sediments in which substantial bacterial populations are present are in themselves surprising. Even more remarkable is the subset of these environments where bacterial populations increase rather than decrease in deeper layers. This situation is not uncommon, however, and occurs for various reasons, such as the resupply of sulphate or the presence of thermogenic methane. The interplay of bacterial and thermogenic processes in deep marine sediments is shown in *Figure 9*.

The environments where bacterial populations increase in deeper layers demonstrate the presence of a deep biosphere in marine sediments and suggest the probable existence of bacteria at much greater depths than have been currently sampled. An extreme version of this situation occurs in gas-hydrate deposits, where not only do bacterial populations and rates of activity increase around the deep gas-hydrate zone but also rates for certain bacterial activities can be even greater at depth than at the near-surface (*Figure 10*, *Table 1*). The increased activity rates include methane oxidation, and because gas hydrates have been estimated to contain twice the global amount of carbon compared to "normal" fossil fuels (Kvenvolden 1988), they represent a globally significant energy source for a deep subseafloor biosphere. However, the bulk of methane in hydrates is also bacterially produced, and an extensive even deeper biosphere must exist to produce this methane.

In the gas-hydrate sediments at Blake Ridge, the principal energy source for the deep production of bacterial methane is acetate, an organic acid associated with thermogenic alteration ($>80\,^{\circ}C$) of buried organic matter as part of the processes of fossil-fuel formation (Cooles et al. 1987). Although temperatures at Blake Ridge are less than $30\,^{\circ}C$, experimental evidence demonstrates the generation of acetate by bacteria at these temperatures (Wellsbury et al. 1997). Thus, it is possible that bacteria obtain energy in deep sediments by catalysing processes previously thought

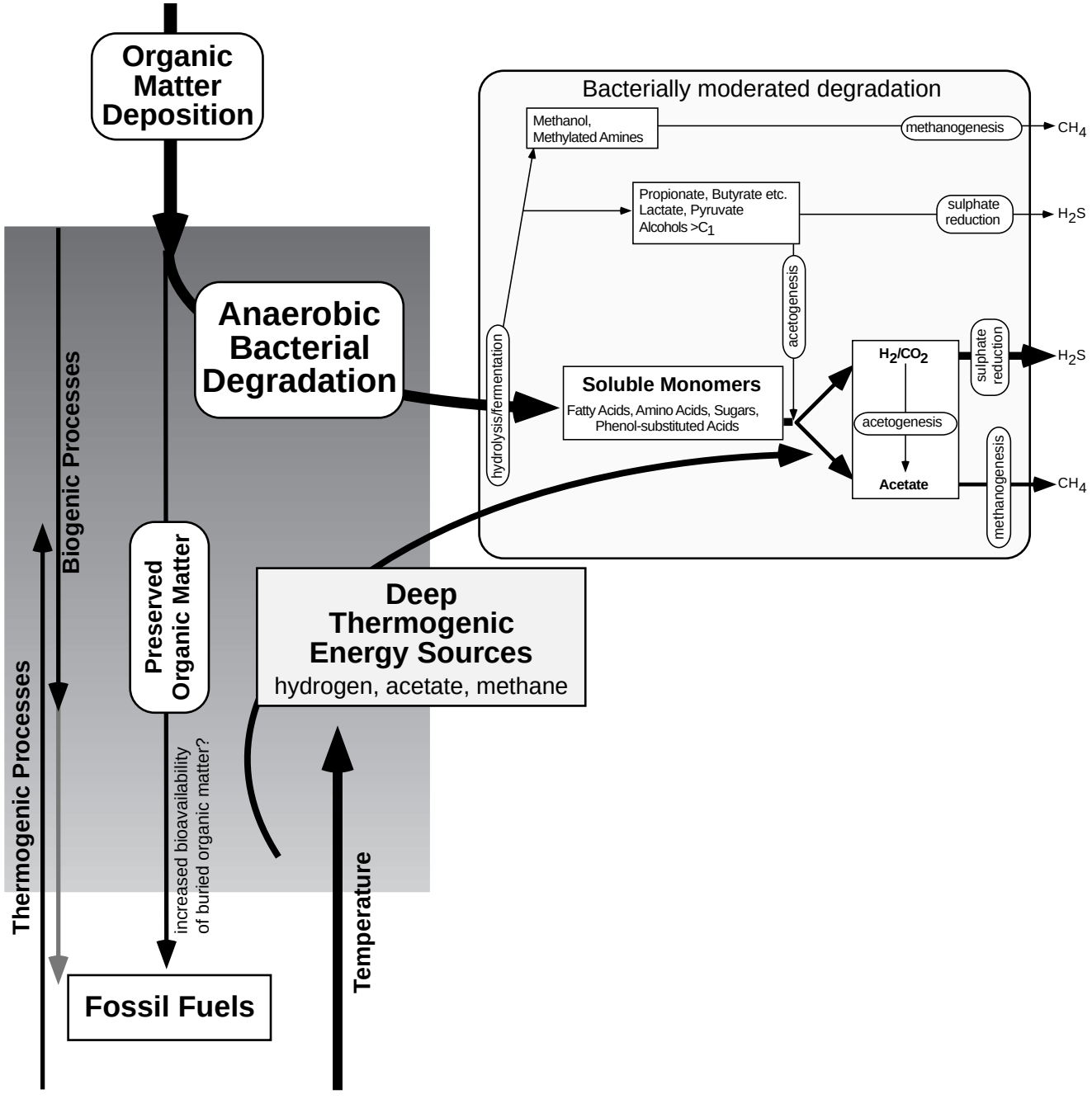

Figure 9 Conceptual model of anaerobic carbon flow in deep marine sediments, showing how increasing temperature during burial may directly stimulate deep bacterial activity and provide bacteria with new deep thermogenic energy sources. Hence, considerable interplay may occur between bacterial and thermogenic processes in deep marine sediments

to be thermogenic and/or by using thermogenic organic-matter breakdown products (*Figure 9*). Because hyperthermophilic (up to 113 °C) and possibly even higher-temperature bacteria are present in subsurface sediments (*Figures 3* and *4*), these processes might continue even to depths where petroleum begins to form; the "oil window" occurs at 100–150 °C (Killops and Killops 1993). In addition, recent results demonstrate that bacteria may be involved in weathering basaltic basement rocks beneath the sediment, which provides a new and possibly even deeper subseafloor habitat (Fisk et al. 1998).

The presence of significant biological activity in the subsurface is contrary to the usual perception of ecosystems on Earth, in which biological activity is concentrated near the surface due to its reliance either directly or indirectly on photosynthetically fixed organic matter. Hydrothermal ecosystems were the first exception to this perception (Karl 1995), although

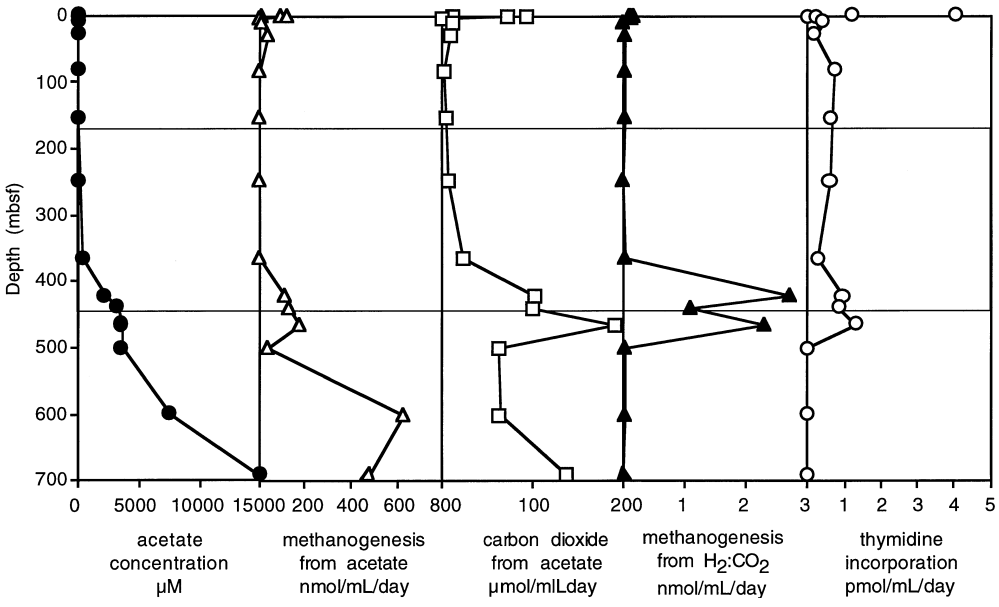

Figure 10 Depth profiles of concentrations of pore-water acetate and rates of bacterial activity in gas-hydrate sediments from Blake Ridge, ODP Leg 164. *Thin lines* denote upper and lower (bottom-simulating reflector) boundaries of gas-hydrate stability field

many of the bacteria and their dependent macroorganisms rely on the metabolic waste product of photosynthesis, oxygen, to exploit energy from high-temperature (up to 380 °C) hydrothermal fluid, with its range of reduced chemicals (sulphides, methane, metals, etc.). Considerable bacterial populations might exist beneath hydrothermal systems (Deming and Baross 1993), and evidence now supports this (*Figures 3* and *4*). If the high-temperature subsurface bacterial populations associated with lateral seawater flow (*Figure 4*) exploit a unique subsurface high-temperature habitat, they could be completely independent of surface photosynthesis. A similar but low-temperature chemoautotrophic ecosystem has been described at depth in Columbia River Basalts in the USA (Stevens and McKinley 1995), where anaerobic rock-weathering reactions produce H_2, which is used by methanogens, acetogens, and sulphate-reducing bacteria. These organisms are the primary producers in a unique photosynthetically independent microbial ecosystem. However, the significance of this H_2-generation mechanism has recently been questioned (Anderson et al. 1998).

Bacteria in the subsurface are diverse and appear to be as responsive as their near-surface counterparts. If energy is available, they can both grow and move quickly (*Figure 7*) and at rates greater than sediment accumulation. Hence, they are not necessarily entombed within their depositing sediment layer. Growth rates, however, decrease rapidly with increasing depth, reflecting the decrease in degradable organic matter. Thus, bacteria in deeper sediments may become restricted to their sedimentary horizon, unless additional energy sources become available. The presence of high bacterial concentrations in discrete sapropel layers demonstrates this situation (*Figure 8*). However, these bacteria are still growing, albeit very slowly; minimum division time is 105 ky. The sapropel results demonstrate the ability of bacteria to obtain energy from old (4.7 mya), deeply buried, and recalcitrant organic matter. A similar situation probably occurs in normal marine sediments; however, in these sediments the recalcitrant organic matter is probably limiting and bacterial populations decrease with depth. Such exceptionally long division times may indicate that subsurface bacteria use energy for maintenance and thus survival rather than growth, and this would help to explain their surprisingly high biomass.

Recent estimates of global bacterial biomass by Whitman et al. (1998) demonstrate that the majority of bacterial biomass occurs in the subsurface, and that the majority of this is in marine sediments. The same authors estimate that subsurface bacteria are also growing very slowly, which is consistent with the sapropel results. Therefore, surprisingly, the majority of bacteria on Earth inhabit the subsurface and in contrast to surface organisms they have a strategy of high biomass and low growth rate that is commensurate with their geological habitat of low-energy flux. However, some subsurface marine environments exist where energy flux is intense, such as gas-hydrate deposits, which enable intense subsurface bacterial activity to occur.

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