# Recent progress in the microbiology of deep-sea hydrothermal vents and seeps

Douglas C. NELSON

*Section of Microbiology - Hutchison Hall - University of California - Davis, CA 95616*
*Fax: (1) 530 752 9014 - e-mail: dcnelson@ucdavis.edu*

Recent findings about microbiology at deep-sea hydrothermal vents and seeps are considered here under three separate headings: (1) endosymbiotic members of the domain Bacteria, (2) free-living mesophilic bacteria, and (3) free-living archael and bacterial thermophiles and hyperthermophiles. Although the headings cover the full spectrum of prokaryotes in these environments, the examples selected are representative rather than exhaustive, with the choices inevitably reflecting personal biases. In these environments symbiotic and free-living prokaryotes can reach impressive biomass densities (Jannasch et al., 1989; Nelson et al., 1991; Hessler et al., 1988; Barry et al., 1997). There is little doubt that symbiont biomass dominates at vents, but at certain seeps, free-living mesophilic bacteria may well be most abundant (McHatton et al., 1996). Our knowledge of thermophilic and hyperthermophilic prokaryotes (archaea plus bacteria) at vents is in an early stage. While an ever expanding evolutionary and physiological diversity of pure culture representatives is being uncovered, the densities of these high temperature prokaryotes venting into ambient deep ocean waters are generally unimpressive (Table 1). Some type of prokaryote-dominated thermophilic "subsurface biosphere", below the mid-ocean volcanic ridges, may be a source of these venting thermophiles. However, almost nothing is known about the density or productivity of microbes there (Kerr, 1997).

## I. Symbiotic Bacteria at Vents and Seeps

All of the symbioses discussed here involve highly specific association of a single type of bacterium with a particular species of host. The general properties of symbiotic sulphur-oxidizing bacteria of deep-sea vents and seeps are reviewed elsewhere (Nelson & Fisher, 1995). Although none of these has been propagated in pure culture, their capacity for fixation of inorganic carbon and oxidation of sulphur compounds are well understood as are a number of adaptive features of specific hosts. Two emerging research areas, focused on crucial interactions of these bacteria with their respective hosts, will be discussed here. The first depends on the recent revelation that there are two fundamentally different ways in which these hosts acquire

Table 1. Cell densities of Bacteria and Archaea at hydrothermal vents.

| Environment | Temp (°C) | Density (cells ml$^{-1}$)[a] | References |
|---|---|---|---|
| Low temperature fluids | < 50 | 5 - 10 x 10$^6$ | Jannasch & Wirsen 1979 |
| | < 50 | < 2 x 10$^6$ | Karl, 1987; Straube et al., 1990 |
| | < 50 | 10$^8$ - 10$^9$ | Corliss et al., 1979 |
| High temperature fluids | 304 | 5 x 10$^5$ | Baross et al., 1984 |
| | 174 - 357 | < 3 x 10$^5$ | Straube et al., 1990 |
| Inside chimney structures | 65 - 80 | 2 x 10$^5$ - 5 x 10$^7$ (cells g$^{-1}$ dry wt.) | Harmsen et al., 1997a |

[a] In some cases the primary data were in the form of particulate DNA or ATP concentrations. These were converted to prokaryotic cell densities using macromolecular percent composition of *Escherichia coli* (Migula, 1895), (see Ingraham et al., 1990).

symbionts. In one pattern, exemplified by vesicomyid clams, symbionts are transmitted vertically from one generation to the next. Here the symbionts are found in abundance only in the gill tissues. Although absent from other host tissues, they have been detected in follicle cells surrounding primary oocytes in *Calyptogena magnifica* Boss & Turner, 1980 and *C. pacifica* (Dall, 1891) (see Cary & Giovannoni, 1993). Further evidence that these bivalves acquire symbionts via eggs stems from the observation that genes encoding small subunit (SSU) ribosomal RNA (rRNA) molecules of symbionts have been successfully amplified from ovarial tissue of three *Calyptogena* species. Perhaps the strongest evidence that this general pattern of inheritance prevails throughout vesicomyids is seen by comparing the phylogenetic trees of hosts and symbionts (Peek et al, 1998). The branching orders in both trees (SSU rRNA for symbionts, cytochrome oxidase for hosts) are virtually identical, with a vanishingly small probability that this host-symbiont congruence arose by chance alone. Only vertical transmission of symbionts is consistent with such

cospeciation. Similar inheritance patterns prevail in the symbionts of *Solemya* spp. found in sulphidic, "vent analog" environments (Cary, 1994; Krueger et al., 1996).

For other chemoautotrophic symbionts, inheritance by the next generation of host is quite different, involving environmental re-infection. It is uncertain whether this is through an infective form of symbiont that is shed from intact associations and is transiently viable outside the host. Alternatively, this transmission may indicate that a particular bacterium capable of sustained autonomous growth can also proliferate to colonize gill or trophosome tissue when it encounters its specific host. The best understood example of this type of inheritance is found in the Vestimentifera. For the giant vent tube-worm, *Riftia pachyptila* Jones, 1981, no DNA could be amplified from eggs with symbiont-specific PCR primers (Cary et al., 1993). Likewise, for the closely related *Ridgeia piscesae* Jones, 1985, no symbiont was detected in ovarian tissue using appropriate probes for in situ hybridization. Among vestimentiferans, phylogenetic analyses make it clear that host and symbiont phylogenies are not convergent. For example, the symbionts of *R. pachyptila* and *Tevnia jerichonana* Jones, 1985 appear to be identical (Laue & Nelson, 1997; Feldman et al, 1997) even though the hosts are extensively diverged. The conclusion is that among a number of vestimentiferans, physical proximity of hosts at the same vents is pivotal in determining symbiont identity while evolutionary proximity of two hosts is not. These data are only consistent with environmental re-infection; therefore, the symbionts of vestimentiferans deserve special culturing efforts. Why a host that is completely dependent on its symbionts for nourishment has adopted this seemingly risky strategy, is certainly an open question.

A second fertile research area in the study of vent bacterial endosymbionts involves homogenization of the symbiont-containing tissues (e.g. gill or trophosome) followed by purification of the symbiont. The symbiont preparations can then be subjected to short term physiological studies, much as one would with pure cultures, varying parameters such as temperature, hydrostatic pressure, the nature of reduced sulphur compound supplied, or the concentration of a particular electron donor or acceptor. Because autotrophy is the hallmark of these symbionts, the variable most often monitored is the rate of $^{14}CO_2$ incorporation into intracellular or acid stable products. Criteria that must be satisfied to have confidence in the conclusions from such studies have been reviewed extensively in the recent literature (Nelson & Fisher, 1995). This approach has, for example, recently yielded conclusions regarding the psychrophilic nature of vent mussel and clam symbionts, their preference for various reduced sulphur compounds, and the benefit of starvation of the intact association for a few days prior to harvesting the symbionts (Nelson et al., 1995; Nelson & McHatton, 1996). This last procedure allows depletion of endogenous reserves of sulphur compounds and renders symbionts more responsive to exogenous energy sources.

Perhaps the most significant questions yet to be answered by this approach focus on whether a particular host is nourished:

(1) By using lytic enzymes (most logically in a vacuole) to consume a significant fraction of its symbionts per unit time. These are then, presumably, replaced by division of the remaining symbionts.

(2) By a relatively stable population of symbionts that over-produce one or a few organic compounds that are "leaked" or excreted to supply the energy and carbon for host cell growth.

If a model system can be found that displays the excretion of a major portion of its fixed carbon in one or a few types of molecules, perhaps in response to particular host factors, this would be strong evidence for the second type of nutritional interaction. Some progress has been made with the tube-worm symbionts (Felbeck & Turner, 1995), but the study of these symbionts is complicated by the physical separation of symbionts from the hydrothermal fluids. In this association, $CO_2$ must be transported from the surrounding seawater via gills and blood to the symbiont replete trophosome tissue. If this occurs via conversion of $CO_2$ to one or more organic compounds as has been suggested (Felbeck & Turner, 1995), it imposes additional difficulties on decisions about which labelled organic compounds might be excretion products, destined for the host, vs. which are in transit to the symbionts, for further modification.

To date the symbionts of *Bathymodiolus thermophilus* Kenk & Wilson, 1985 and *Calyptogena magnifica* have proven to be ideal experimental systems for short term physiological studies with purified symbionts. They respond with strongly enhanced $CO_2$ incorporation when stimulated with appropriate sulphur compounds and sustain their fixation rates for several hours (Nelson & Fisher, 1995; Nelson & McHatton, 1996) but are available only infrequently from deep-sea hydrothermal vent expeditions. Several of the much more accessible bivalve/sulphur-bacteria associations, e.g. *Solemya reidi* Bernard, 1980, *S. velum* Say, 1822 and *Calyptogena kilmeri* (Bernard, 1974) have been tested recently while searching for an ideal model system. Partially purified symbionts from these associations have so far proved intractable (Nelson & McHatton, 1996 and unpublished) because the incorporation rates were low or extremely variable or because they were not responsive to sulphur compound stimulation. Among the many "vent analogue", sulphide-rich, sediment communities of diverse marine environments, there exist numerous other symbiont/bivalve associations (Fisher, 1990). These should be surveyed for an appropriate and readily accessible model system using the criteria set forth elsewhere (Nelson & Fisher, 1995).

## II. Mass assemblages of free-living mesophilic prokaryotes at vents and seeps

This section provides three separate examples of recently discovered, free-living, bacterial populations that dominate certain niches at vent or seep ecosystems. The niches and bacteria are, to varying degrees, novel ones, and these discoveries have been achieved using a combination of approaches including molecular, physiological, and enzymological characterizations of dominant natural populations, enrichments of certain bacteria under conditions that mimic vent environments, and studies with pure culture. As additional specific habitats and populations are characterized by this suite of approaches, general themes may emerge.

The first specific example - vacuolate, nitrate-accumulating bacteria of the genus *Beggiatoa* that dominate at certain seeps - is covered in more detail elsewhere (McHatton et al., 1996). These extremely wide, gliding, sulphide-oxidizing, autotrophic, and filamentous prokaryotes (Nelson et al., 1989; McHatton et al., 1996) can also dominate vent environments (Jannasch et al., 1989). The common adaptive features of these *Beggiatoa* spp. are the possession of a massive central vacuole and the intracellular accumulation of nitrate at levels approaching 0.2 M. Hydrogen sulphide, an abundant respiratory electron donor in vent ecosystems, can be oxidized by these bacteria in a novel and efficient way using the internal nitrate as an electron acceptor. Oxygen, the normal electron acceptor of non-vacuolate *Beggiatoa* spp., would penetrate only a few millimetres into the dense *Beggiatoa* mats, but the use of nitrate permits 10 to 20 cm thick mats to consume all of the surrounding sulphide.

There has been much recent interest in and speculation about a microbe-dominated deep-subsurface biosphere and its relationship to the 60,000-kilometer-long system of mid-ocean ridges that circles the earth. Perhaps the most dramatic evidence to date of this presumed connection was afforded by 1991 submersible dives on the East Pacific Rise in the region between 9°47' and 9°51'N. These coincided, by chance, with a major eruption of the mid-ocean ridge. White particles of elemental sulphur-rich biogenic debris were suspended by rapidly venting fluids to an altitude of 50 m above the bottom creating a "virtual blizzard" in the water column, and the fallout of this biogenic snowstorm was observed 100 m down current from the axial summit caldera (Haymon et al., 1993). The long thin filaments that dominated the output of these "snowblower vents" (Nelson et al., 1991) were presumed, based on morphology, to be sulphur-oxidizing archaea (Haymon et al., 1993). According to one scenario they could represent permanent residents of the subsurface hydrothermal biosphere transiently proliferating when eruptions generated these same hot, sulphide-rich conditions over large stretches of the surrounding seafloor (Haymon et al., 1993). Very recent studies with a model laboratory system (Taylor and Wirsen,

1997) offer an alternative explanation for the snowblower vents. In nearshore seawater distant from any vent source, these researchers found a novel, sulphide-tolerant, vibrioid microbe that elaborates a "filamentous sulphur" morphology indistinguishable from that which dominated the snowblower vents. This mesophilic microbe is, based on its niche, almost certainly bacterial rather than archael. Its high capacity for $CO_2$ fixation, its extraordinary ability to oxidize sulphide and its high sulphide tolerance - well above that of any other mesophilic bacterium isolated from hydrothermal vents - suggest that its proliferation following the 1991 eruption was a result of the extraordinarily high concentrations of sulphide present. A year after the eruptions, much smaller populations of bacteria forming this same "filamentous sulphur" were observed around warm, sulphide-rich vent openings. They, too, were mesophilic, $CO_2$-fixing, and highly sulphide-tolerant but were being actively grazed by large swarms of copepods (Nelson, unpublished). Thus, they may contribute a significant part of the non-symbiotic productivity at sulphide-rich vents in general, but their importance at 9°N was only realized under the conditions that allowed the rapidly growing bacteria to transiently surpass the capacity of grazers.

A final example of a vent-dominating, mesophilic bacterium reflects the interplay between molecular characterization of in situ microbial populations and pure culture studies. Pele's Vents are located approximately one kilometre below sea level near the summit of the Loihi Seamount. Their chemistry, reflective of a typical midplate rather than a plate boundary hydrothermal vent, is quite acidic and high in dissolved $CO_2$ (ca. 300 mM), iron (ca. 1 mM), and $CH_4$ (ca. 7 µM). By contrast dissolved sulphide is absent or very low in concentration. Bacterial mat material collected from these vents yielded a total of 48 separate SSU rDNA clones that represented 12 distinct OTUs (operational taxonomic units), the two most abundant of which accounted for 73% of all the clones (Moyer et al., 1994). Although the sequences of these rRNA genes showed similarity with diverse groups in the domain Bacteria, evolutionary distances were great enough to preclude genus assignment in almost all cases (Moyer et al., 1995). Very recently a novel, gradient-dwelling, neutral pH, $Fe^{2+}$-oxidizing bacterium was cultured from several freshwater environments (Emerson & Moyer, 1997). The three clones characterized by SSU rRNA sequence analysis were virtually identical to each other and fell within a novel lineage in the gamma subgroup of the Proteobacteria. Surprisingly (Emerson & Moyer, 1997), the only other sequence coincident with this cluster was the second most abundant OTU from Pele's Vents, comprising 25% of all the rDNA clones. Although it is risky to infer physiology from phylogeny, the SSU rRNA data and the vent fluid characteristics both point to iron oxidation as a dominant nutritional process at Pele's Vents. The virtual absence, both from culture collections and the SSU rRNA data base, of

neutral pH, iron-oxidizing bacteria also emphasizes the essential role of continued culturing studies in parallel with characterization of natural populations.

III. Diversity and Importance of Thermophilic Prokaryotes at Vents

Early reports of turbid bacterial suspensions issuing from low temperature hydrothermal vents (Corliss et al., 1979) raised the prospect that this was the output from a dense hydrothermal subsurface biosphere. Subsequent assessment of prokaryote abundances in cool and very hot hydrothermal flows (Table 1) showed unimpressive densities less than $5 \times 10^6$ cells per ml, the density of bacteria required to marginally support suspension feeding bivalves (Page et al., 1990). Although it appears that this type of diffuse prokaryotic output is insufficient for supporting vent food chains, an episodic higher density output of thermophiles or mesophiles from subsurface chambers might be in the form of large clumps, sheets, or mats. Thus, the snapshot approach (Table 1) could easily underestimate the overall importance of these outflows. It was initially assumed that the dominant prokaryotes of "snowblower" vents were dramatic transient evidence of this phenomenon. Our current assumption that these were mesophilic populations downplays the trophic role of thermophiles. The density of bacteria and archaea within the walls of chimneys can be significantly higher (Harmsen et al., 1997a; Table 1). Based on both culturing and group specific rRNA/rDNA probes many of these chimney-associated prokaryotes were shown to be thermophiles (Harmsen et al, 1997a,b). Very recent data also indicates that abundant bacterial epibionts on alvinellid polychetes may include thermophiles (Cary et al., 1997).

Based on a representative suite of thermophiles prokaryotes cultured from shallow and deep-sea hydrothermal vents (Table 2) a few generalizations can be made about their niches and trophic roles. Many of them are obligate anaerobes that require, or are enhanced by, elemental sulphur (S°) and $H_2$. The former is a partial oxidation product of the sulphide abundant in many vent fluids, and the latter can be produced via abiotic reactions between reactive basalt and water (Stevens & McKinley, 1995). The concentrations of these and a few other inorganic electron donors and acceptors, e.g. $Fe^{2+}$, $Fe^{3+}$ and $NO_3^-$, are likely to set the upper limit to thermophilic prokaryotic productivity by lithoautotrophs at vents. Many of the other metabolic types encompassed by Table 2, for example anaerobic fermenters and heterotrophs, depend on pre-formed organic matter to fuel their energy metabolism and biosynthesis. Especially at non-sedimented hydrothermal vents the source of this organic matter is likely to be the lithoautotrophic thermophiles just mentioned (see also Table 2). A cycling of these prokaryotes through lethal temperature regimes would generate the requisite dilute organic solutions and particulate organic matter. As the use of molecular

**Table 2.** Representative thermophilic prokaryotes cultured from marine hydrothermal vents.

| Domain | Genus/ Species | Optimum (°C) | Energy Metabolism | Electron Donors, Acceptors | Refs. |
|---|---|---|---|---|---|
| BACTERIA | | | | | |
| | *Thermotoga* spp. | 80 | anaerobic fermenter | S° or $S_2O_3^{2-} \rightarrow H_2S$ (as sink for inhibitory H2) | 1, 2 |
| | *Thermosipho africanus* Huber et al., 1989 | 75 | anaerobic fermenter | S° or $S_2O_3^{2-} \rightarrow H_2S$ (as sink for inhibitory H2) | 3, 4 |
| | *Aquifex pyrophilus* Huber et al., 1992 | 85 | strict lithoautotroph | $H_2$, $S_2O_3^{2-}$ S° as donors $O_2$, $NO_3^-$, S° as acceptors | 5 |
| ARCHAEA | | | | | |
| Euryarchaeota | *Thermococcus* spp. | 80-90 | anaerobic fermenter | S° $\rightarrow H_2S$ (some obligate) | 6-9 |
| | *Archaeoglobis* spp. | 82 | anaerobic; mixotroph or facultative lithoautotroph | $H_2$ or organic donor $S_2O_3^{2-}$, $SO_4^{2-} \rightarrow H_2S$ | 10, 11 |
| | *Methanococcus* spp. | 85 | obligate anaerobe | $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ | 12, 13 |
| | *Pyrococcus* spp. | 95-100 | anaerobic fermenter | S° $\rightarrow H_2S$ (as sink for inhibitory $H_2$) | 14-16 |
| Crenarchaeota | *Staphylothermus marinus* Fiala et al., 1986 | 85-92 | anaerobic heterotroph | S° $\rightarrow H_2S$ (obligate) | 17 |
| | *Desulfurococcus* spp. | 85-90 | anaerobic fermenter | S° $\rightarrow H_2S$ (as sink for inhibitory $H_2$) | 18 |
| | *Pyrodictium* spp. | 97-105 | obligate anaerobe lithoautotroph/fermenter | $H_2$ or organic donor S° or $S_2O_3^{2-} \rightarrow H_2S$ | 19 |

References: 1 Huber et al., 1986; 2 Jannasch et al., 1988a; 3 Ravot et al., 1996; 4 Huber et al., 1989; 5 Huber et al., 1992; 6 Neuner et al., 1990; 7 Gonzalez et al., 1995; 8 Kobayashi et al., 1994; 9 Godfroy et al., 1996; 10 Stetter, 1988; 11 Burggraf et al., 1990; 12 Jones et al., 1983; 13 Zhao et al., 1988; 14 Fiala and Stetter, 1986; 15 Jannasch et al., 1992; 16 Erauso et al., 1993; 17 Fiala et al., 1986; 18 Jannasch et al., 1988b; 19 Pley et al., 1991.

probes in these high temperature vents becomes more sophisticated, as our catalogue of pure cultures of vent thermophiles expands, and as our ability to spatially characterize the thermal regimes at the time of microbial sampling takes on greater precision, the trophic relationships between thermophilic archaea and bacteria should become clearer. Long term continuous monitoring of specific vents, including the ability to preserve well characterized water samples, will advance our appreciation for the spectrum of bacterial outputs from hydrothermal vents.

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