

The ecology of ectosymbiosis at a Mid-Atlantic Ridge hydrothermal vent site

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Abstract: The phylogenetic approach to microbial ecology has radically altered our view of bacterial diversity in nature. Analysis of ribosomal RNA sequences have revealed an extensive number of previously unrecognized microorganisms. This has led to an expectation of complex microbial communities in nature although virtually nothing is known about their structure. It has been shown previously that in a hydrothermal vent habitat, where food webs depend on prokaryotic primary production, the attached microbial community consists largely of only one bacterial phylogenetic type (phylotype) as indicated by the dominance of a single 16S rRNA sequence. The main part of its population occurs as an ectosymbiont on the dominant animals, the shrimp *Rimicaris exoculata*, where it grows as a monoculture within the carapace and on the extremities. However, the same bacteria are also the major microbial component of the free-living attached community as determined by quantitative nucleic acid hybridization. The retrieved sequence is distinctly different from all studied chemoautotrophic endo- and ectosymbioses from hydrothermal vents and other sulfidic habitats, which all cluster within the gamma-Proteobacteria. This contribution demonstrates that the shrimp epibionts are part of one of two novel groups within the epsilon-Proteobacteria. All other phylotypes reported within these groups occur as epibionts or free-living bacteria at hydrothermal vents and may be of worldwide distribution.

Introduction

Hydrothermal vents on the ocean floor are very unusual ecosystems. They resemble oases in the surrounding desert of the deep sea, occurring in irregular intervals along the spreading zones of the oceanic plates, often hundreds of kilometers apart (for a review, see FISHER 1990). While the deep-sea environment generally depends on the sparse rain of photosynthetically fixed carbon from surface waters, hydrothermal vents reach high productivity through local energy sources. These are primarily geothermally reduced sulfur compounds and methane emanating in warm or hot anoxic fluids from the basalts. Mixing with the surrounding oxygenated ocean water results in a cocktail which contains the ingredients for chemoautotrophic primary productivity. This bacterial process utilizes energy contained in reduced chemical compounds for carbon dioxide fixation and uses oxygen as an electron sink. Indeed, the energy output at hydrothermal vents can be large enough to nourish

communities whose productivity has often been compared to that of tropical rain forests.

At Pacific vents, this high productivity has been concluded to be largely associated with endosymbiotic bacteria of sessile tube worms and bivalves (WIRSEN et al. 1993). These form extremely dense clusters around the vents and may reach growth rates which are among the highest known (LUTZ et al. 1994). However, as these communities entered the textbooks as typical vent fauna, hydrothermal vents on the Mid-Atlantic Ridge (MAR) were discovered. These gave a radically different picture. Most of the sites are dominated by highly mobile decapod shrimp of the family Bresiliidae. The extremities and inside of the carapace of these shrimp are densely overgrown with bacteria, but they apparently lack endosymbionts (VAN DOVER 1995). While symbiont-bearing mussels are abundant at some MAR sites, tubeworms, clams, and alvinellid polychaetes are absent and are, with the exception of the latter, known only from cold seep environments in the Atlantic (VAN DOVER 1995).

Extremely large and stable populations of these bresiliid shrimp (Fig. 1) were observed over several years at TAG and Lucky Strike, the two better-studied vent sites at the MAR (VAN DOVER 1995). They cluster on the surfaces of black smokers and mounds, both formed by solid sulfides which precipitate from the reduced vent fluids upon contact with oxygenated deep water. The shrimp are highly mobile but typically stay close to the surfaces, where they often form a layer several specimens thick with an estimated density of up to 25 000 to 50 000 specimens per m² (Fig. 1) (SEGONZAC et al. 1993, VAN DOVER 1995). Five species have been identified to date, one of which, *Rimicaris exoculata*, vastly dominates.

One of the most unusual features of these shrimp is the extremely dense bacterial populations growing on their anterior extremities and on the inner carapace wall (GEBRUK et al. 1993, VAN DOVER et al. 1988, WIRSEN et al. 1993). The gill chamber formed by the carapace is enlarged and filled with extended setae originating from the mouthparts; these setae are densely overgrown and have been termed bacteriophores (SEGONZAC et al. 1993). At least two morphological types of bacteria can be identified: filaments of varying thickness which are attached with a holdfast (WIRSEN et al. 1993) and simple rods which lie on the cuticle (GEBRUK et al. 1993, SEGONZAC et al. 1993). These bacteria possess autotrophic capabilities as judged from activity measurements of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the CO₂-fixing enzyme of the CALVIN-BENSON cycle, in extracts of carapace scrapings. They are inferred to gain energy from the oxidation of reduced sulfur compounds (WIRSEN et al. 1993).

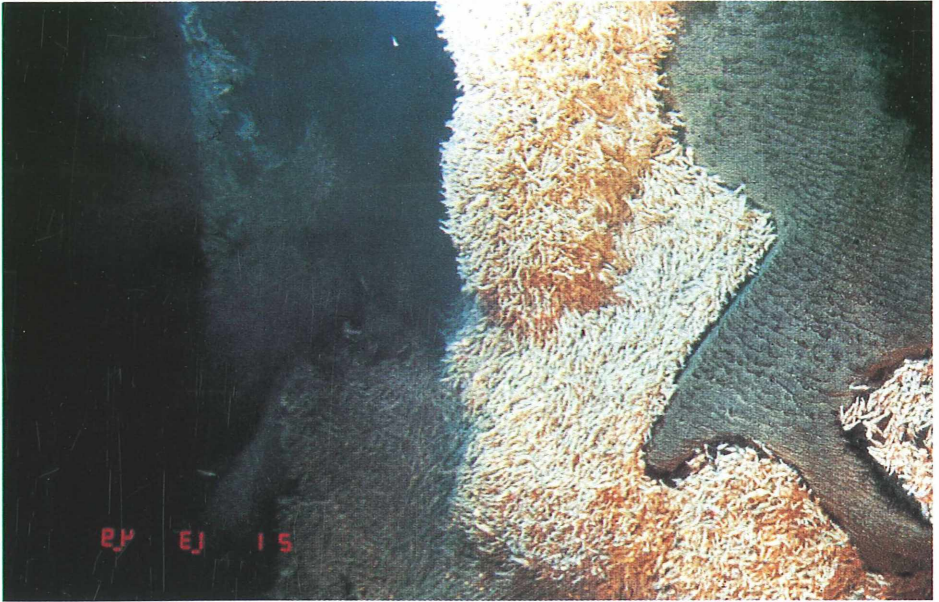


Fig. 1: Extremely large aggregations of the Mid-Atlantic Ridge hydrothermal vent shrimp *Rimicaris exoculata*. The animals cluster on the surface of a chimney wall. Each of these animals carries a massive monoculture of epibiotic bacteria which has been shown to be the dominant microbial species in the habitat (Photo from DSRV "Alvin" by P. RONA, Rutgers University and G. THOMPSON, Woods Hole Oceanographic Institution).

The ecology and phylogenetic relationships of these epibiotic bacteria have been examined recently (POLZ and CAVANAUGH 1995). Given the strikingly different animal communities at Pacific and Atlantic vents, yet the mass occurrence of bacteria-invertebrate associations at both, we investigated the epibacteria in respect to (i) their specificity for the shrimp host, (ii) their phylogenetic relationships, and (iii) their overall environmental importance in the surface-associated community (POLZ and CAVANAUGH 1995). All known sulfur-oxidizing ecto- and endosymbioses are monospecific and fall within two closely related clusters within the gamma-Proteobacteria. Thus, unraveling the specificity and phylogenetic identity was important in deciding whether the epibionts represent a fouling community or true symbionts and enabled us to address the question of their representation in the surface community. This surface community as opposed to the plankton microbial

community, is presumed to be the primary food source for higher trophic levels at the MAR. The dominating macrofaunal element, the shrimp, either graze bacteria off their own body or from the sulfide surfaces and do not possess typical filter-feeding appendages (VAN DOVER 1995, and C.L. VAN DOVER pers. comm.). Furthermore, no significant populations of filter-feeding animals have been described from the MAR vents.

We review our recent findings on the specificity, phylogeny, and ecology of the shrimp ectosymbionts (POLZ and CAVANAUGH 1995) and determine their relationships with newly identified bacterial sequences from other hydrothermal vents in the deep sea. The phylogenetic approach to the study of microbial ecology as first introduced by PACE and colleagues (PACE et al. 1986) was used since it allows the investigation of unculturable microorganisms even when sampled in such remote environments as the hydrothermal vents.

The phylogenetic framework

The phylogenetic approach to the study of microbial communities avoids bias introduced by culture-based enumeration which typically only detects <1 % of the naturally occurring bacteria (AMANN et al. 1995). As a first step, the diversity of a given bacterial community is assessed. This is done by assuming that libraries of 16S ribosomal RNA genes are representative for the native organisms from which the genes originated. Representative clones can then be sequenced and the information used (i) to reconstruct the phylogenetic relationships of the organisms, and (ii) to design oligonucleotide probes specific for the given sequence. These probes are an excellent tool for the quantification of the relative importance of bacteria in nature. When hybridized to total nucleic acids extracted from an environmental sample, their signal intensity can be compared to that of probes specific for larger taxonomic units, such as all Bacteria or Archaea. Results are expressed as a percentage and should be interpreted as a hybrid of abundance and activity of the bacteria in question when targeted against DNA and RNA. Since the bacteria described in this study could not be cultured and their identification relies on genetic information, the term "phylotype" is used instead of species or strain.

A detailed description of the experimental outline is given elsewhere (POLZ and CAVANAUGH 1995). Briefly, samples were collected with the deep submersible "Alvin" during a cruise in 1993 to the Snake Pit site along the MAR. To analyze the epibiont community, 16S rRNA genes were amplified by the Polymerase Chain Reaction (PCR) using universal primers (LANE 1991). The PCR product was sequenced directly (HULTMAN et al. 1989) to determine if a single sequence predominated in the DNA obtained from the

epibionts. In addition, the PCR product was cloned, and a partial sequence determined for 26 randomly picked clones. Subsequently, the epibiont sequence was aligned with sequences from representative closely and distantly related bacteria and their detailed phylogenetic relationships determined by distance and maximum parsimony methods (POLZ et al. 1994, POLZ and CAVANAUGH 1995). To confirm the source of the dominant sequence, fluorescent oligonucleotide hybridization (DELONG et al. 1989) using a sequence-specific and a negative control probe with two introduced mismatches were performed on sections of the whole shrimp carapace. The same probes in combination with Bacteria-, Archaea-, and Eukarya-specific probes (STAHL and AMANN 1991) were then used in quantitative slot blot hybridizations on nucleic acids extracted from various surfaces sampled from the shrimp environment (POLZ and CAVANAUGH 1995).

The specificity of the epibiotic community

Despite the morphological differences among the epibionts (Fig. 2), a single 16S rDNA sequence was obtained from the shrimp (POLZ and CAVANAUGH 1995). Direct sequencing yielded a clean, unambiguously readable sequence indicating that one type vastly dominated in the PCR product (POLZ and CAVANAUGH 1995). This suggested the presence of a single bacterial phylotype on the shrimp and was also observed in the analysis of the clones, where 23 of 26 contained the identical sequence, while the remaining 3 were unreadable. However, since some bacterial cells are known to lyse with low efficiency (MOORÉ et al. 1994) and the PCR is prone to contamination and bias in product ratios (POLZ and CAVANAUGH 1995 unpubl. manuscript), it was necessary to test if this dominance in the PCR product was reflected in the composition of the epibiont population. In situ hybridizations on sections of shrimp carapaces were performed using a shrimp-epibiont-specific probe and a negative control probe (POLZ and CAVANAUGH 1995). A probe complementary to all Bacteria (STAHL and AMANN 1991) 16S rRNAs served as a positive control.

The in situ hybridizations and epifluorescence microscopy confirmed that the dominating sequence originated from the shrimp epibionts (POLZ and CAVANAUGH 1995). Furthermore, it showed that the different morphotypes contain identical 16S rRNA sequences and thus represent a single phylotype. Pleomorphy is common among bacteria and is usually associated with changes in growth conditions. On the shrimp body, however, filaments and rods occur side by side. Whether their different morphologies represent different growth stages, microvariation in the physico-chemical environment, or simply very closely related strains with identical 16S rDNA cannot be determined. Never-

theless, the association displays all signs of a symbiosis (SMITH and DOUGLAS 1987), i.e., a stable and specific association between a bacterial group and the shrimp host.

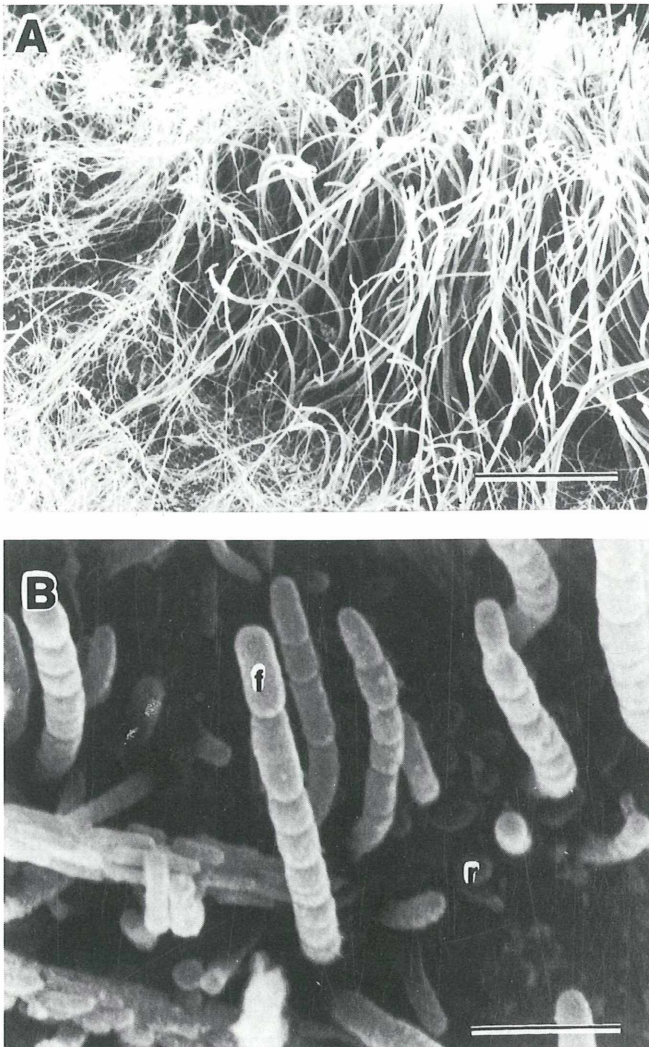


Fig. 2: Bacterial epigrowth on the anterior extremities of the hydrothermal vent shrimp *R. exoculata*. Scanning electron microscopical micrographs showing (A) the dense epigrowth on "bacteriophage" setae of a shrimp appendage, and (B) the different morphotypes, the filaments (f) and rods (r). (Scale bars: A, 100 μ m; B, 1 μ m).

Epibiotic bacteria can be found on a number of marine animals (SIEBURTH 1975). However, their specificity and composition has only been investigated in two additional cases, the sediment-inhabiting nematode *Laxus* sp. (POLZ et

al. 1994) and the hydrothermal vent polychaete *Alvinella pompeijana* (HADDAD et al. 1995). *Laxus* was shown by direct sequencing and oligonucleotide probing to carry a single phylotype with uniform morphology on its body and was the first example of extreme specificity in an epibiotic association (POLZ et al. 1994). Superficially, the epibiotic community of *A. pompeijana* resembles the shrimp ectosymbionts, with filamentous morphotypes dominating. However, in a preliminary analysis on a single specimen, 32 clone families were recovered from PCR products, indicating a much higher diversity and lower specificity of this association (HADDAD et al. 1995). Since *A. pompeijana* is quite abundant at some Pacific vent sites, it will be interesting to determine whether this association is a symbiotic one as well.

The phylogenetic relationships of the epibionts

The 16S rRNA sequence was used to infer phylogenetic relationships between the shrimp ectosymbionts and other known symbiotic and free-living bacteria (POLZ and CAVANAUGH 1995). It fell within the epsilon-subdivision of the Proteobacteria and was the first symbiotic species distinctly separate from all other known ecto- and endosymbiotic sulfur-oxidizing bacteria (POLZ and CAVANAUGH 1995), which form two loosely associated clusters within the gamma-subdivision of the Proteobacteria (DISTEL et al. 1994, POLZ et al. 1994). The epsilon-subdivision spans a wide range of evolutionary distances but only few genera have been identified to date. However, these display a few common characteristics, such as association with animals (albeit often parasitic), microaerophily, and distinct sulfur-metabolic pathways. Since the completion of the analysis of the shrimp ectosymbiont relationships (POLZ and CAVANAUGH 1995), epsilon-Proteobacteria sequences have been found in clone libraries derived from a bacterial mat from vents at Loihi seamount (MOYER et al. 1995) and from an epibiotic community of the hydrothermal vent polychaete *A. pompeijana* (HADDAD et al. 1995).

A phylogenetic analysis including these sequences from the Loihi microbial mat and the *Alvinella* epibionts reveals the relationships between these newly recovered epsilon-Proteobacteria phylotypes and the shrimp ectosymbionts (Fig. 3). The shrimp bacteria cluster with the four epsilon bacterial clones obtained from the alvinellid polychaete (HADDAD et al. 1995) and form a monophyletic clade with these in both distance and parsimony analysis, as shown by high bootstrap values (Fig. 3). However, the shrimp sequence is relatively distant from all other sequences in this group, with the closest relative, clone ALVRRDA, reaching only 90.8 % identity (Tab. 1). The epsilon clones recovered from Loihi all display an extremely high degree of similarity (MOYER et al. 1995) (Tab. 1) and form a monophyletic group which

is not closely related to the epibiotic sequences from shrimp and polychaetes (Fig. 3, Tab. 1). The sulfur-oxidizing, gradient organism *Thiovulum* sp. is associated with the clade of the Loihi microbial mat clones (Fig. 3).

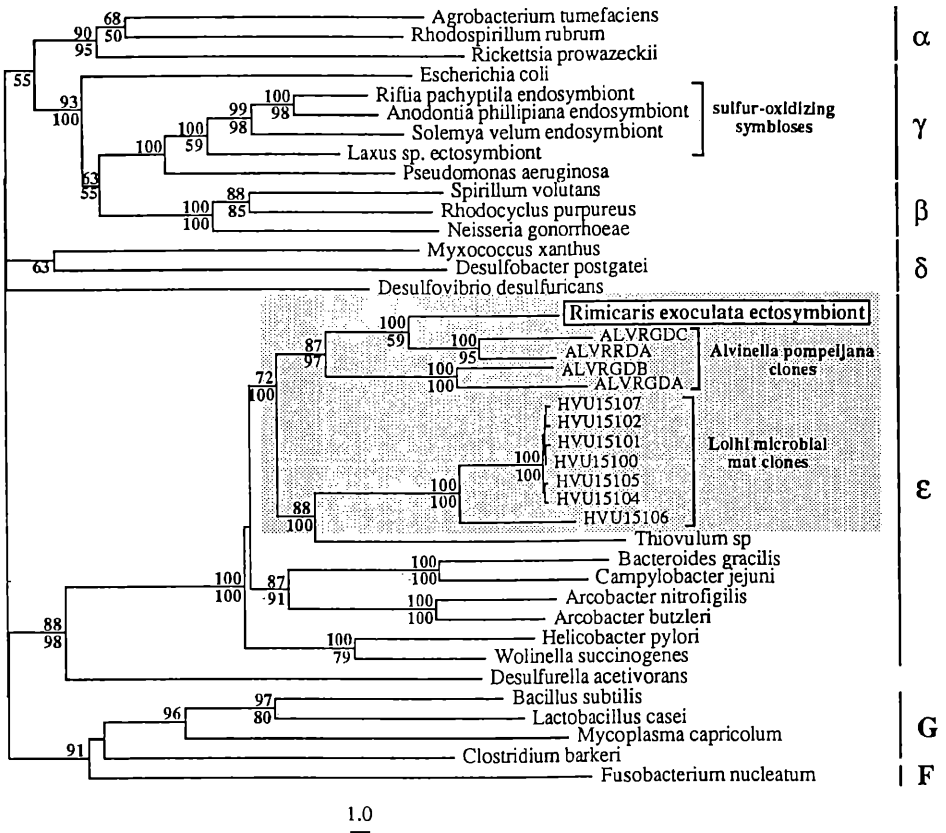


Fig. 3 A 16S rRNA-based phylogenetic distance tree showing the relationships of the *R. exoculata* epibiotic bacteria to known free-living and symbiotic bacteria within the five subdivisions (a, b, g, d, e) of the Proteobacteria. Representatives of the Gram-positive phylum (G) and the Fusobacteria (F) were included as outgroups to the Proteobacteria. Bootstrap values higher than 50/100 are shown. Upper values were obtained in a distance analysis, lower values in a parsimony analysis which gave an essentially identical tree topology (data not shown). The alignment included 1018 sequence positions. Most sequences were obtained from the Ribosomal Database Project (LARSEN et al. 1993); the *R. exoculata* sequence, *A. pompeijana*, and Loihi microbial mat clones can be retrieved from Genbank under the accession numbers U29081, L35520 to L35523, and U15100 to U15107, respectively. Details of the methodology are given in POLZ et al. (1996) and POLZ and CAVANAUGH (1995). Scale bar represents percent nucleotide difference per sequence position.

Tab. 1: Identity matrix for 16S rDNA sequences^a of the *R. exoculata* ectosymbiont, epsilon-Proteobacteria clones recovered from hydrothermal vent environments^b and *Thiovulum* sp.

Organism	% Identity to												
	1	2	3	4	5	6	7	8	9	10	11	12	
1. <i>R. exoculata</i> ectosymbiont													
2. ALVRRDA	90.8												
3. ALVRGDA	87.0	87.9											
4. ALVRGDC	88.9	95.4	88.7										
5. ALVRGDB	86.0	88.3	93.3	88.6									
6. HVU15100	86.6	85.9	84.5	85.3	84.0								
7. HVU15101	86.5	85.9	84.4	85.2	83.9	99.9							
8. HVU15102	86.6	85.9	84.7	85.2	84.2	99.4	99.3						
9. HVU15104	86.6	85.9	84.6	85.3	84.1	99.2	99.1	99.4					
10. HVU15105	86.6	86.1	84.6	85.4	84.2	99.3	99.2	99.3	99.3				
11. HVU15106	86.6	85.2	82.9	84.7	83.4	95.0	94.9	94.8	94.8	94.9			
12. HVU15107	86.3	85.7	84.3	85.0	83.9	99.3	99.2	99.3	99.0	99.0	94.6		
13. <i>Thiovulum</i> sp.	82.6	82.9	81.7	82.7	82.3	85.2	85.1	85.1	85.3	85.2	84.9	84.9	

^a Identity values (%) were calculated from an alignment of the 16S rDNA sequences which included 1018 sequence positions.

^b clones beginning with ALV obtained from an epibiotic community of the hydrothermal vent polychaete *A. pompeijana* (Haddad et al. 1995) and clones beginning with HVU obtained from a microbial mat at Loihi seamount (Moyer et al. 1995).

The placement of these shrimp ectosymbionts and environmental clones within the epsilon-Proteobacteria points to a worldwide distribution of this group in hydrothermal vent environments, as they have been recovered from both Atlantic and Pacific vents. *R. exoculata* appears to be abundant all along the MAR (VAN DOVER 1995), the Loihi seamount is situated in the Hawaiian island chain, and *A. pompeijana* is common at vents along the East Pacific Rise. Although only these three examples have been found to date, it appears that geography does not determine relatedness in this group of epsilon-Proteobacteria, as the sequences derived from the Atlantic shrimp and the Pacific polychaetes are more closely related to one another than to the microbial mat sequences.

The environmental importance of the ectosymbionts

To assess the overall importance and distribution of the shrimp ectosymbionts at the MAR site, quantitative oligonucleotide slot blot hybridizations on total nucleic acids extracted from different environmental sources were performed (POLZ and CAVANAUGH 1995). Probes specific for the three domains Bacteria, Archaea, and Eukarya yielded information on the relative representation of these groups, and the shrimp ectosymbiont-specific probe was used to

quantify the proportion of ectosymbiont to total community nucleic acids (POLZ and CAVANAUGH 1995). Sources of nucleic acids were all ectosymbiont-bearing body parts of ten shrimp specimens and eight randomly selected sulfide blocks sampled from the Snake Pit hydrothermal vent, whose surfaces were scraped to a depth of 1 to 2 mm (Tab. 2).

Tab 2: Quantitative analysis of the contribution of ectosymbiont-specific nucleic acids to the total microbial community^a.

Nucleic Acids	replicate slots	Amount Measured by Hybridization Probes		
		Bacteria Specific	Ectosymbiont	Ratio (%) ^d
Shrimp-associated ^b	5	586.8±59.7	572.9±12.5	97.6
Sulfide-associated ^c	3	64.0±1.3	39.2±1.9	61.3

^a modified after Polz and Cavanaugh (POLZ and CAVANAUGH 1995b). Values were obtained by quantitative slot blotting of parallel setups with **Bacteria**, Ectosymbiont-Specific, and negative control probe, respectively. Samples and standards were hybridized on the same membrane and quantified by Phosphor Image analysis. The values are reported as mean ng nucleic acids • standard error.

^b ng nucleic acid per individual, pooled nucleic acids from 10 individuals.

^c ng nucleic acid per g substrate, pooled nucleic acids from eight scraped sulfide surfaces.

^d ratio of hybridization signal of the ectosymbiont-specific probe to that of the **Bacteria** probe (×100).

The results showed that the shrimp ectosymbionts play a dominating role at this vent site (POLZ and CAVANAUGH 1995). They contribute over 60 % of the detectable bacterial nucleic acids on the sulfide surfaces, indicating a large and very active population. This is supported by scanning electron microscopical observations that detected the typical filamentous bacteria on sulfide surfaces (POLZ and CAVANAUGH 1995, WIRSEN et al. 1993). The quantification also confirmed the conclusions from the in situ hybridizations that only one phylotype grows on the shrimp body, since the signal of the specific probe was almost 100 % that of the Bacteria probe (Tab. 2) (POLZ and CAVANAUGH 1995). Neither Archaea nor Eukarya were detectable on the sulfide surfaces (POLZ and CAVANAUGH 1995), which parallels results obtained at a Pacific vent site analyzing lipid biomarker molecules (HEDRICK et al. 1992). Thus, it is possible that at hydrothermal vents Archaea are

confined to hot and/or anaerobic niches. This suggests that Archaea do not occur abundantly in aerobic microbial communities, as has been found recently for plankton communities (DELONG 1992, DELONG et al. 1994, FUHRMAN et al. 1992).

The predominant role of the shrimp symbiosis at the Snake Pit vent is illustrated by estimating cell numbers from the amount of nucleic acids recovered from the different sources (POLZ and CAVANAUGH 1995). Assuming that an average bacterial cell contains 15 fg of total nucleic acids of which 23 % are 16S rRNA (NEIDHARDT 1987), a rough estimate of cell numbers can be calculated from the quantitative probing (Tab. 3). Although ribosome content is linked to growth rate, it is thought that variation in actively growing cells in the environment is only 5- to 10-fold (KERKHOF and WARD 1993, POULSEN et al. 1993). Thus, the bacterial biomass associated with the shrimp body would considerably exceed that of the free-living surface community on the sulfides in the areas of greatest shrimp densities (Tab. 3). These areas are likely to reach highest chemoautotrophic productivity, since the shrimp cluster around actively venting fluids which carry reduced sulfur compounds. It is also likely that the planktonic community under direct influence of the vents is less important than the shrimp ectosymbionts. It would take approximately 425 to 850 l of water to equal the biomass associated with the 25 000 to 50 000 shrimp per m² estimated at these vents (Tab. 3). Considering that chemical gradients which supply the energy for most of the primary productivity at hydrothermal vents are very steep because of both rapid mixing and chemical oxidation of sulfide, the planktonic community under direct influence of the vents may be comparatively small.

Tab. 3: Comparison of estimates of bacterial numbers for various vent habitats.

Estimated no. of bacteria ^a	Source
8.5 × 10 ⁶	single shrimp
2.1 to 4.2 × 10 ¹¹	25,000 to 50,000 shrimp per m ²
4.9 × 10 ⁸	per m ² sulfide surface (depth of 1 mm)
5.0 × 10 ⁵	per ml vent water from Pacific vents

^a Values for shrimp and sulfides obtained from quantitative probing assuming 15 fg of 16S rRNA per average bacterial cell which is approximately 23% of the total nucleic acids, value for Pacific vent water from (KARL et al. 1980).

The large population of the epibiotic phylotype growing on the shrimp may have a positive effect on the free-living population of the same phylotype and may even trigger its dominance. A constant rain of cells that are detached from the shrimp body may serve as an inoculum to the surface community and may give the epibiotic phylotype an advantage over non-symbiotic bacteria living on the sulfide surfaces if they occupy a similar niche. This can essentially be viewed as adding an additional positive term to the growth equation of the epibiotic phylotype when growing on the sulfides and may lead to their extreme dominance even when free-living on the sulfide surfaces.

Nutritional aspects of the shrimp symbiosis

The role of the ectosymbionts in the nutrition of the vent shrimp has been the subject of debate. Based on examination of the gut contents and functional morphology of the anterior appendages, VAN DOVER et al. (1988) suggested that the shrimp feed off the sulfide surfaces by picking up particles and ingesting them. This view has subsequently been challenged by a number of authors (GEBRUK et al. 1993, SEGONZAC et al. 1993); however, little data have been put forward in support of a reliance on the ectosymbionts as a food source and the issue awaits more detailed investigations.

The shrimp may actually depend on both sources of nutrition, the ectosymbionts and the free-living surface community. Their behavior seems ideally suited for their ectosymbionts to reach high productivity. They constantly move around in the mixing zones of reduced vent and oxygenated ocean water, which continuously supplies the bacteria with high concentrations of energy substrates. Similar adaptations have been described for other sulfur-oxidizing nutritional symbioses (CAVANAUGH 1994). Furthermore, the stable carbon isotopic signature values of the shrimp resemble those of the ectosymbionts rather than those of the free-living bacteria on the sulfides (C.L. VAN DOVER, pers. comm.), indicating that the shrimp graze off their body. On the other hand, quantitative probing on pooled gut contents of 50 shrimp revealed a ratio of ectosymbionts to total bacteria more similar to the sulfide surfaces than the ectosymbionts (pers. observ.) and the guts are usually packed with sulfide particles (VAN DOVER et al. 1988). Currently we carry out a more detailed investigation of the nutritional sources of the vent shrimp using stable isotopes and genetic markers.

Analogous to the Pacific, symbiotic organisms dominate hydrothermal vents on the MAR. However, ectosymbiosis appears to give rise to more complex and indirect interactions than endosymbiosis. Like the endosymbionts, the ectosymbionts probably reach rapid growth rates as indicated by high rRNA content (POLZ and CAVANAUGH 1995). If the shrimp eat bacteria

from within their carapace, these will be a reliable internal food source. In addition, if the shrimp graze the attached microbial community on the sulfides, they will have access to two sources of nutrition. The bacteria falling off the shrimp bodies may also play an important role, since they help in replenishing and inoculating the free-living community stressed by grazing. Thus, the shrimp would act as a gardener planting new crops on a field. This dual mode of food supply may enable the shrimp to reach the extremely high population densities observed at the MAR by preventing overgrazing of either the free-living community or the ectosymbiont population.

Conclusions

The MAR hydrothermal vent shrimp show that symbiotic associations stand at the base of hydrothermal vent communities. While in endosymbioses dominant at Pacific vents positive links between host and symbionts are very direct, they may be more indirect between ectosymbionts and shrimp at MAR vents. The proposed seeding mechanism may ensure ample food supply for the large shrimp populations and may also trigger the highly unusual dominance of a single phylotype in this environment. Whether this mechanism can be found in other facultative symbioses where the symbionts possess a free-living state is currently being investigated.

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