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Article

TEM Observations of Chemosynthetic Bacteria in the Deep-sea Hydrothermal Vents and Seep Organisms

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Abstract : Symbiosis of chemoautotrophic bacteria with the members of hydrothermal vent and cold seep communities in the deep-sea were examined by histology using transmission electron microscopy; *Bathymodiolus* spp. from Sagami Bay, the Iheya Ridge and the North Fiji Basin; and *Ifremeria nautilei* from the North Fiji Basin. Two species of *Bathymodiolus*, each from Sagami Bay and the Iheya Ridge harbored methane-oxidizing symbionts within their gill tissues. Vent gastropod *Ifremeria nautilei* from the hydrothermal vents of the North Fiji Basin housed two types of symbionts; one sulfur-oxidizing type and the other methane-oxidizing type. The occurrence of chemosynthetic symbionts in these organisms were expected beforehand based on the ecological observations of their habit. The other members of these groups from world oceans and the recent advances in the symbiosis of the vent and seep communities were reviewed.

Key words : symbiosis, benthos, hydrothermal vent, bivalves, gastropod, cold seep.

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1. Introduction

Endosymbiotic associations of bacteria with eukaryotic hosts are widespread in nature. Chemoautotrophic and methanotrophic bacteria have been added to the collection of bacterial endosymbionts (Felbeck 1981; Cavanaugh *et al.* 1981, 1987). The entry of nonpathogenic or parasitic bacteria into host cells largely relies on the phagocytic ability of the cells (Smith 1979). This is the very reason why the great majority of hosts are phagotrophic feeders. De Burgh and Singla (1984) first found phagocytic activity in the gill epithelial cells of an exosymbiont-bearing hydrothermal vent limpets from the Juan de Fuca Ridge. Southward (1986) reported the phagocytic incorporation of exosymbiotic bacteria in the gill epithelial cells of several thyasirid bivalves. In both cases phagocytozed bacteria had rapidly undergone destruction by lysosome fusion, thus stable endosymbiotic associations could not be established.

The chemolithoautotrophic and methanotrophic symbiosis are associations between bacterial symbionts and marine invertebrate hosts. It appears that the symbionts in all of the described chemoautotrophic associations use some form of reduced sulfur as an energy source, although there is no a priori reason not to suppose that other types of chemoautotrophic symbionts may yet be discovered. In 1981 Felbeck reported on the presence of high activities of several enzymes of the Calvin-Benson cycle and bacterial sulfur metabolism in the trophosome of *R. pachyptila*, and Cavanaugh *et al.* (1981) reported that this tissue was packed with large numbers of intracellular prokaryotic cells. These two papers represented the first demonstration of chemoautotrophic bacterial symbiosis with a marine invertebrate. In 1986 the first documented methanotrophic symbiosis was reported from a mussel (*Mytilidae* gen. sp.) of the hydrocarbon seeps in the Gulf of Mexico (Childress *et al.* 1986).

Bacterial endosymbionts using reduced sulfur compounds as energy sources and fixing CO_2 by means of the Calvin-Benson cycle have been implicated as the major source of

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primary production around the deep-sea hydrothermal vents. These bacteria are found in the gills of several bivalves and within the trunk of the vestimentiferan tube worms that live near the vents (Cavanaugh 1983). Since the initial discovery, such symbioses have been found in a variety of other taxa (Cavanaugh 1985; Felbeck *et al.* 1981) as well as a variety of other habitats (Schweimanns and Felbeck 1985; Paull *et al.* 1984; Kennicutt II *et al.* 1985) characterized by the availability of both reduced sulfur compounds and O_2. Shortly after the initial discovery of these symbioses, investigators began to look for symbiosis based on other reduced compounds found in some of these environments. This paper presents here evidence of sulfur and methane-based symbioses between hydrothermal vent and cold seep animals and intracellular bacteria.

2. Materials and methods

Mussels (Photo 1) were sampled during Dives #481 and #542 from the Okinawa Trough and the Minami Ensei Knoll (Fig. 1, for the detailed explanations of these sites, see Hashimoto *et al.* 1990) by "SHINKAI 2000". The provannid gastropod *Ifremeria nautilei* (Photo 2) were collected during Dive #STARMER10 of the French submersible Nautile, and Dives #77 and #80 of "SHINKAI 6500" on a central rift system in the North Fiji Basin at a depth around 1,980 m (Fig. 2). Gill samples (Table 1)

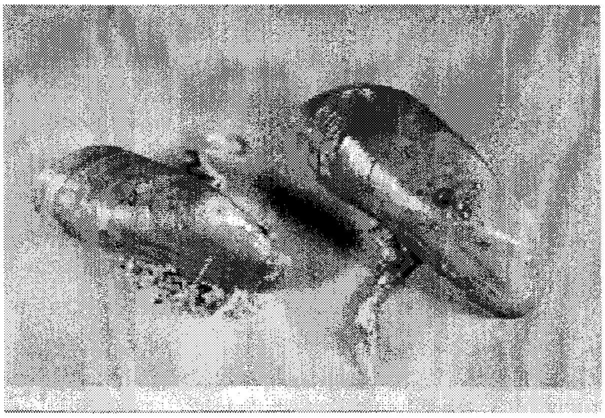

Photo 1. Elongated type of medium-size mussel, *Bathymodiolus* sp. They occur both in Calpytogena Site and Pyramid Site.

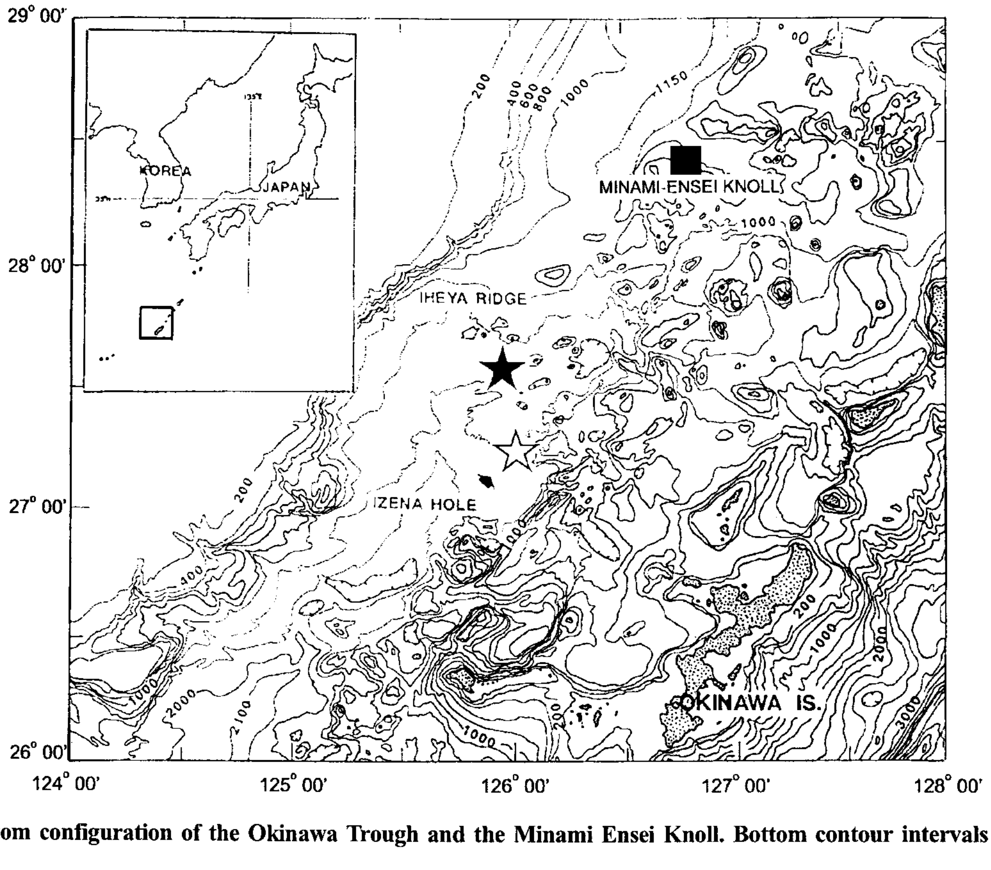

Fig. 1. Bottom configuration of the Okinawa Trough and the Minami Ensei Knoll. Bottom contour intervals are 200 m.

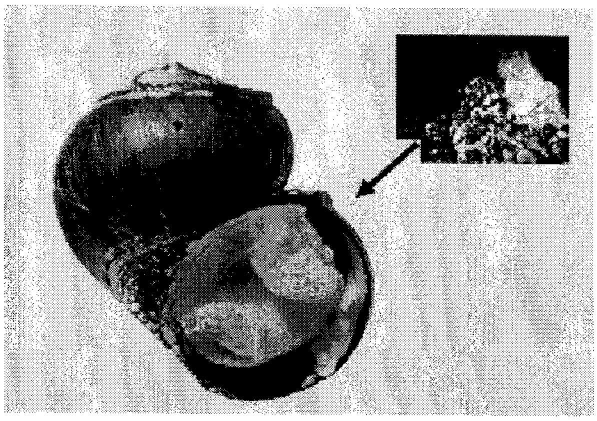

Photo 2. The provannid gastropod *Ifremeria nautilei* from the North Fiji Basin.

were fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer at pH 7.4. After rinsing in the same buffer they were post-fixed in osmium tetroxide (final concentration 1%). Specimens were dehydrated in a graded ethanol series followed by propylene oxide, embedded in Epon 812, and sectioned for light and transmission electron microscopy (T.E.M.). The sectioned material was stained with Toluidine Blue for light microscopy and by uranyl acetate and lead citrate for transmission microscopy. Jeol 100CX transmission electron microscope was used for observations.

3. Results

Ifremeria nautilei of North Fiji Basin

Transmission electron micrograph of the gill of *Ifremeria*

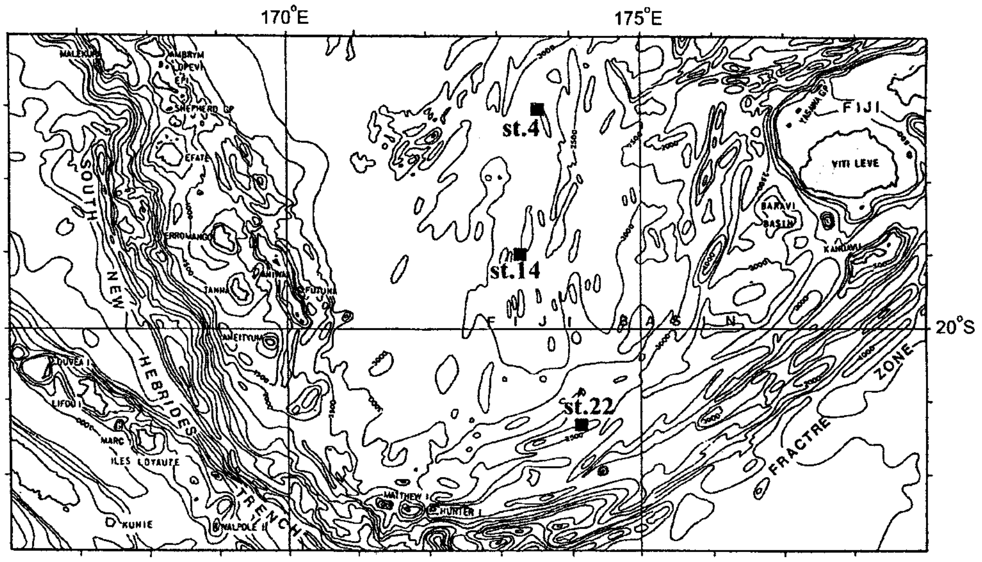

Fig. 2. Detailed bathymetrical map of the North Fiji Basin at a depth around 1,980 m.

Table 1. List of samples observed by transmission electron microscopy (TEM) and light microscopy.

Station or Dive No.	Vessel or Submersible	Date	Locality	Position	Depth	Specimen
#481	SHINKAI 2000	17 May 1990	Pyramid Site Iheya Ridge	27°33.03'N 126°58.15'E	1400 m	*Bathymodiolus* sp.
#542	R/V Tansei Maru	26 May 1991	Minami Ensei Okinawa Tr.	28°23.5'N 127°38.5'E	700 m	*Bathymodiolus* sp.B
STA10	NAUTILE	05 July 1989	North Fiji B.	16°58.67'S 173°54.96'E	1975 m	*Ifremeria nautilei*
#77	SHINKAI 6500	07 Sep. 1991	North Fiji B.	16°58.67'S 173°54.96'E	1975 m	*Ifremeria nautilei*
#80	SHINKAI 6500	11 Sep. 1991	North Fiji B.	16°59.46'S 173°54.87'E	1965 m	*Ifremeria nautilei*

nautilei showed a row of epithelial cells with high densities of symbiotic slender bacteria (Photo 3). The bacteria were packed densely within microvilli-fringed bacteriocytes (Photo 4), especially at the apical part of the bacteriocytes. Large lysosome-like organelles were found localized almost at the basal part of the bacteriocytes (Photo 3).

Two types of symbionts were found inside the same bacteriocytes. One is rod type, membrane-bound bodies approximately 1.1 by 3.9 μm (Photo 4). The second type is of comparable size to the type I, but rather stout in outline containing stacks of parallel membranes which are strikingly similar to those reported from free-living bacteria which utilize methane (Photo 5). The membrane-stacked bacteria were inclined to be localized toward the basal membrane.

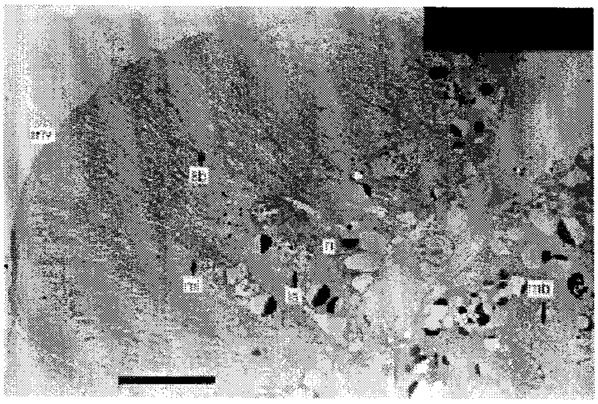

Photo 3. TEM micrograph of the bacteriocytes in the gill tissue of *Ifremeria nautilei* from the North Fiji Basin. Ls: lysosome-like structure; mb: methanotrophs; mi: mitochondria; mv: microvilli; n: nucleus of bacteriocyte; sb: sulfur oxidizing symbionts. scale bar: 10 μm.

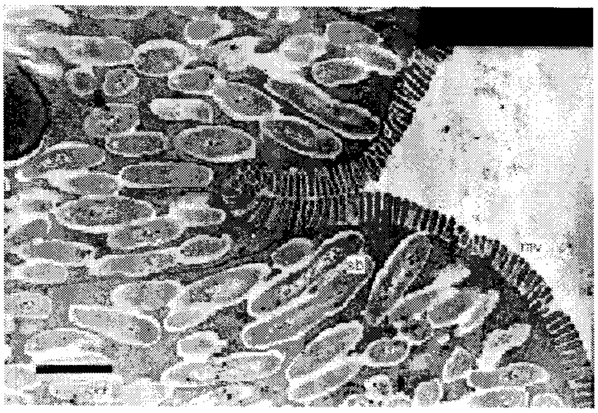

Photo 4. TEM micrograph of the rods near the surface of the microvilli-fringed bacteriocytes in the gill of *Ifremeria nautilei* from the North Fiji Basin. mv: microvilli; sb: surfur-oxidizing symbiont. scale bar: 2 μm.

Both symbionts have Gram-negative type cell walls. Among the two types of symbionts, sulfur-oxidizing bacteria were predominant. In a rough estimate, membrane-stacked methane bacteria amounted to 5% or less of the symbiont population.

Empty cavities suggesting exocytosis were also observed at the apices of bacteriocytes (Photo 5). Dividing stages can be observed (Photo 5). Both types of symbionts reproduce by transverse binary fission. Dividing forms of methane bacteria were less frequent than those of sulfur-oxidizing bacteria.

The endocellular bacteria of the gill resemble in structure and dividing mode, the sulfur and methane-oxidizing bacteria found in *Alviniconcha hessleri* (Endow and Ohta 1989).

Bathymodiolus sp. of the Minami-Ensei Knoll

The gill structure of the mussels are similar to those of symbiont-containing gills of other vent and seep mussels in that they are large, thick and fleshy (Photo 6). Light microscopy and ultrathin transverse-sections through a gill filament revealed it to be composed of a unicellular thin wall lining a central blood sinus. Four different types of gill cells were recognized in the gill filament wall. 1) Ciliated cells having frontal, latero-frontal and lateral

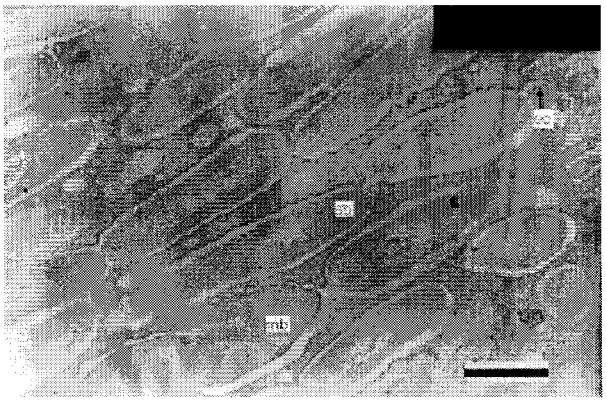

Photo 5. High magnification of slender-rod sulfur-oxidizer and membrane-stacked methane-oxidizer of the bacteriocyte of *Ifremeria nautilei*. Note the connecting canal to the outside of the cell and dividing stage can be observed. ec: empty cavity; mb: methanotrophs; sb: sulfur-oxidizing symbiont. scale bar: 1 μm.

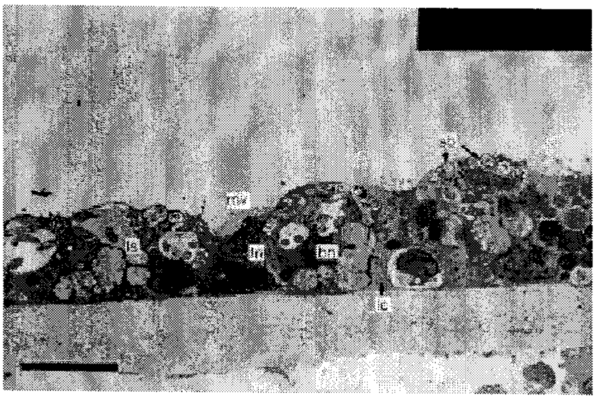

Photo 6. Gill filaments of *Bathymodiolus* sp. from the Minami Ensei Knoll. Transverse section through one face of gill filament showing bacteriocytes and intercalary cells. Lysosome-like structure with the bacteria are visible (ls). Bn: bacteriocyte nucleus; Ic: intercalary cell; In: intercalary cell nucleus; mv: microvilli; sb: symbionts. scale bar: 10 μm.

ciliation and containing high concentrations of mitochondria and dense crystals of unknown substance. They are situated to the distal of the gill filament in cross-sectional view. 2) Mucus cells among the ciliated cells, especially abundant at the distal edge, where they appear to be full of dense droplets of mucus containing abundant endoplasmic reticulum. 3) Voluminous cells containing dense concentrations of symbiotic bacteria. They occupy the major part of the gill filament. The bacteriocytes decrease in thickness from the distal to the proximal part of the gill filament. Sometimes they are intervened by the following cells. 4) Thin intermediate ciliated cells (intercalary cells) separated by the bacteriocytes and goblet mucus cells of the non-ciliated distal edge; these cells are enlarged at their basal part, which is occupied by a large nucleus, and bear cilia and a fringe of microvilli at their external pole. Mitochondria and endoplasmic reticulum are also present in this intercalary cells.

TEM examination of the bacteriocytes along the major central portion of the gill filament revealed large but moderate number of symbiotic bacteria and subcellular inclusions including cell envelops typical of Gram-negative bacteria (Photo 6 and 7). The bacteriocyte of this species is devoid of microvilli in the portion facing seawater. All the symbionts appeared intracellular, in specialized gill bacteriocytes, in vacuoles surrounded by a peribacterial membrane either singly or in groups of two or more. Sometime the free surface of the bacteriocytes are verrucose due to the underlying symbionts.

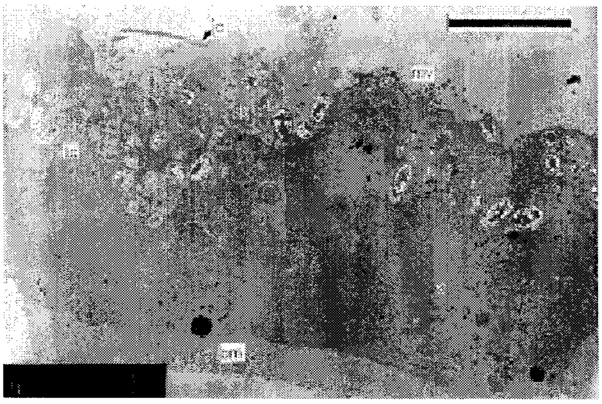

Photo 7. TEM micrograph of the bacteriocyte in the gill tissue of the same individual of the *Bathymodiolus* sp. from the Minami Ensei Knoll. bm: basal membrane; c: cilia; mv: microvilli; Ic: intercalary cell; mv: microvilli. scale bar: 10 μm.

The symbiotic bacteria are seemingly variable in size and ultrastructure misleading into three categorization, i.e., 1) coccoid-shaped cells, about 0.8 to 1.2 μm in longer diameter, containing bundles or stacks of complex intracytoplasmic membranes, 2) rod-shaped cells, about 3.2 to 4.4 μm in longer diameter, also containing stacks of complex intracytoplasmic membranes and 3) uncommon very long shaped bacteria cells attaining to 7.6 μm. However, this is the artefact depending on the orientation of the thin sections, and actually the symbiotic bacteria consist of one sausage-shaped type.

The intracytoplasmic membranes seen in symbionts are reminiscent of those seen in several bacteria, including type I methane-oxidizer (Photo 7).

Generally the bacteria were located at the very surface of the gills. Rod-shaped symbionts seem to be inclined to lying parallel to the free surface of the bacteriocytes. This is probably of particular advantage for gas exchange to the symbionts. Numerous lysosome-like structure beneath the bacterial zone showed lysosomal resorption of the bacteria (Photo 6). This is another potential way of transfer of organic material from the bacteria to their host.

They store irregular polygonal inclusions bodies within their bodies. The symbionts lying near to the free surface of the bacteriocytes are apt contain much vacuoles.

Dividing symbionts were rarely observed. One of the reason is, simply, due to the limited number of symbionts within a bacteriocyte, probably 2 orders of magnitude less than those of the other symbiont-bearing vent organisms.

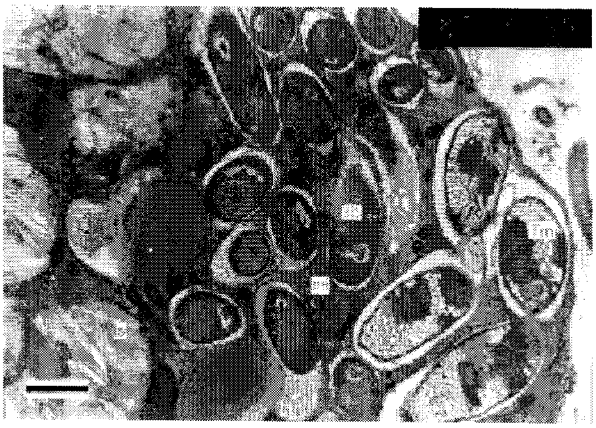

Photo 8. TEM micrograph of the bacteriocyte of the gill tissue of the *Bathymodiolus* sp. from the Iheya Ridge. Stacked internal membranes are typical of type I methanotroph (Tm). ls: lysosome-like structure; mi: mitochondria; sb: symbiont bacteria; va: vacuoles. scale bar: 1 μm.

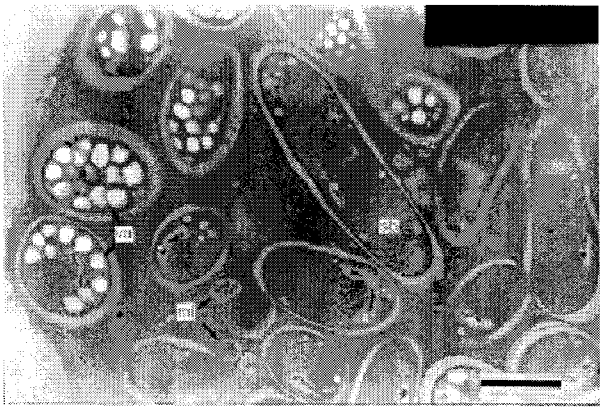

Photo 9. TEM micrograph of the transverse section of the bacteriocyte of the same individual of *Bathymodiolus* sp. from the Iheya Ridge. mi: mitochondria; sb: symbiont bacteria; va: vacuoles. scale bar: 1 μm.

Bathymodiolus sp. of the Iheya Ridge

The gill of this species are composed of numerous filaments with abundant ciliation on the lateral and frontal edges. The gill filaments are held in place by ciliary tufts on their lateral faces as is typical in Filibranchia. The bulks of the lateral faces of above and this species are lined by large bacteriocytes which sometimes alternate with symbiont-free intercalary cells. The bacteria-bearing cells decrease in thickness from the distal to the proximal portion of the gill filament.

TEM observation of bacteriocytes devoid of microvilli large but moderate number of intracellular symbionts in vacuoles (surrounded by a peribacterial membrane) within the bacteriocytes (Photo 8). The bacteriocytes of this mussel house fewer bacteria, and with only one to three bacteria visible per vacuole in cross section as compared with *B. thermophilus* from the 13°N vent field on the EPR (Fiala-Medioni *et al.* 1986), the bacteriocytes of *B. thermophilus* are larger than present species and contain thousands of bacteria per cell, and many bacteria clustered in vacuoles in *B. thermophilus*. Slightly oblique transverse section of gill filament showed that the host cells contain coccoid and rod Gram-negative bacteria.

Symbionts are coccoid or short rods ranging from 0.7 to 2.5 μm in longer diameter in coccoid form and 1.8 to 4.0 μm in longer diameter in rod-shaped type (Photo 9).

Symbionts are concentrated at the apical pole of the bacteriocytes. Beneath the bacterial zone and the basal part of the bacteriocyte is a well-developed nucleus surround by endoplasmic reticulum and mitochondria. Dense lipid droplets and abundant lysosome-like organelles which contribute to the granular aspect of the bacteriocyte. Stacks of intracytoplasmic membranes, typical of type I methanotrophs, are visible in many of these symbionts. The fact that symbionts are very close to the gill surface would facilitate CH_4 uptake from seawater. However, all the bacteriocytes of the mussels examined lack microvilli on the outer facets, and they seem to be not suited for direct uptake of methane and/or dissolved organic material.

4. Discussion

Ifremeria nautilei of North Fiji Basin

The present study showed that bacteriocytes of *Ifremeria nautilei* were packed with two types of Gram-negative symbionts. The distribution pattern of the bacteria in *I. nautilei* is similar to those of the gill symbionts in *Alviniconcha hessleri* (Stein *et al.* 1988; Endow and Ohta 1989). Most of the symbionts seem to be fully enclosed in the bacteriocytes. Fairly frequent fusion of putative lysosome membranes with peribacterial membranes also supports the internalization of symbionts within the host cells. In order for an endosymbiotic association to be stable, many problems must be solved by both sides of the symbionts and hosts. Among these, it is essential for symbionts to effectively escape from lysosomal attack of host cells (Southward 1986; Giere and Langheld 1987; Endow and Ohta 1989). To keep away from areas of high lysosomal activity to areas of low or no digestive activity is one of the most simple way of settlements (Bannister 1979; Giere and Langheld 1987).

Abundant rod-shaped symbionts must be sulfur-oxidizing bacteria by its morphology. Another one with complex intracytoplasmic membrane stacks must be methane-oxidizing nature. Other than cyanobacteria, complex intracytoplasmic membrane stacks have been known to occur in very limited groups of bacteria, namely phototrophs, nitrifying bacteria and methanotrophs. The membrane stacks of the *I. nautilei* symbionts most resemble those of the type I methanotrophs.

Cavanaugh *et al.* (1987) reported two types of symbionts in the same bacteriocyte of the seepage mussel of the Florida Escarpment. Based on the co-occurrence of type I methanotrophic enzyme activities and type I intracytoplasmic membrane stacks of methanotrophs, they suggested that one of the mussel symbionts was a methane-oxidizer.

It is not so common in nature that multicellular hosts harbor more than one kind of symbionts at the same time in the same cell. However, it would be expected that microbes with unique requirement for energy or nutrition, such as chemoautotrophs, methanotrophs can possibly live together with vast range of organisms without severe competition (Endow and Ohta 1989).

The distribution pattern of methane bacteria within the bacteriocytes along with their possible uneven distribution in host individuals can be explained by acquisition of guest symbionts from the outer environment or by the multiplication of hidden symbionts. It is considered that the methane bacteria are guest symbionts coming into the bacteriocytes of *I. nautilei* from the exterior, being brought into the *I. nautilei* with water current introduced for respiration.

I. nautilei may alternatively rely on their symbionts principally for detoxification of sulfide thus allowing them to graze upon epilithic bacteria close to the vents. However, gastropods that graze on sulfur bacteria at shallow-water vents (Stein 1984) do so without benefit of sulfide detoxification by bacterial symbionts.

Elemental sulfur stored by bacteria within the gills of symbiont-containing gastropods represents an intermediate product of sulfide oxidation that may serve as an energy storage which can be oxidized to produce energy in the absence of ambient sulfide.

Bathymodiolus

The ciliation of the frontal and lateral faces of the filaments of this deep-sea mytilids does not differ, in basic plan, from that of littoral species. This observation, together with previous data describing a functional feeding groove at the base of the lamellae and well developed labial palps, as well as gut-content analysis (Le Pennec and Hily 1984; Le Pennec and Prieur 1984) confirms the ability of this bivalve to capture particulate matter. Another possible source of nutrition for these mussels is uptake of dissolved organics, and Fiala-Medioni *et al.* (1986) have demonstrated uptake of labeled amino acids from relatively low environmental concentrations.

However, the lateral faces of the filament are quite different in these hydrothermal and cold seep mussels; they are made of epithelial cells separated by sparse ciliation and microvilli. Histological and ultrastructural sections clearly revealed a large number of endocellular symbiotic bacteria in the gill tissue, except for the two small frontal and apical areas. The filament is principally composed of cells containing dense concentrations of bacteria perfectly integrated in the gill tissue (Fiala-Medioni 1984; this study). This is in complete agreement with the hypothesis of the trophic importance for this mytilid of its associated bacteria, which are able to fix carbon-dioxide by utilization of hydrothermal and/or seep chemicals. Similar metabolic pathways have been described for a number of bivalves and pogonophoran worms living in rich sulfide and/or hydrocarbon habitats (Felbeck and Somero 1982; Felbeck *et al.* 1983).

There are at least seven (Galapagos, Mariana, Florida, Gulf of Mexico, Sagami Bay, Okinawa Trough, Kaikata Seamount) different species of mussel currently known to contain symbiotic chemoautotrophic symbionts. Apart from the present (two?) species, one mytilid species at the Florida Escarpment and the species from hydrocarbon seeps in the Gulf of Mexico have been reported to contain methanotrophic symbionts (Childress *et al.* 1986; Cavanaugh *et al.* 1987). Type I methanotrophs have the ribulose monophosphate cycle and incorporate carbon from CH_4 into organic compounds (Higgins *et al.* 1981). This symbiosis between a methanotrophic bacteria and an animal host is potentially able to derive a large fraction of its energetic and carbon needs from the consumption of the reduced single carbon compound CH_4.

The methane consumption is limited to the gills of these animals and is apparently due to the abundant intracellular bacteria found there. Methane consumption is depend on the availability of oxygen and is inhibited by acetylene. The consumption of methane by these mussels is associated with a dramatic increase in oxygen consumption and carbon dioxide production. As the methane consumption of the bivalve can exceed its carbon dioxide production, the symbiosis may be able to entirely satisfy its carbon needs from methane uptake.

The case for methane oxidation being the major energy and carbon source for the symbionts of the seep mussel appears unequivocal, and always lighter δ13C values throughout the mussel soft tissues indicates that methane may be the primary carbon source for the mussel as well (Childress *et al.* 1986).

However, the fact is that reduced sulfur compounds are also present in its environment (Childress *et al.* 1986) and the oxidation of reduced sulfur compounds has been suggested as the major energy source for the symbionts of the closely related vent mussel, *B. thermophilus* (Rau and Hedges 1979; Felbeck *et al.* 1981; Belkin *et al.* 1986). However, as Cavanaugh (1985) noted, there is little direct evidence that reduced sulfur compounds are the major energy source for *B. thermophilus* symbionts. This situation has prompted us to compare these symbiosis with respect to their energy sources.

The mussel can live on reduced carbon and nitrogen compounds synthesized by its associated bacteria as well as on dissolved organic compounds present in seawater. Mussels can feed on particulate material including bacteria aggregates present in the advected currents of the vents (Jannasch and Wirsen 1979; Comita *et al.* 1984). Particulate organic material is especially important in the outer limits of a hydrothermal community, where it provides a complementary food source allowing the mussel to survive. Mussels collected from more peripheral clumps in the hydrothermal vent field had a significantly heavier δ13C than mussels collected from central sites (Fisher *et al.* 1988). The ecological distribution of hydrothermal vent mytilids which can survive at the periphery of the vent sites (Hessler and Smithey 1983; Smith 1985) as well as observations showing *Bathymodiolus* sp. to be one of the last vent species to survive after the subsidence of hydrothermal vents (Laubier and Desbruyeres 1984) suggest that this mytilid is undoubtedly less exclusively dependent on its endocellular bacteria than *Calyptogena* spp.

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References

Bannister, L.H. 1979. The interactions of intracellular Protista and their host cells, with special reference to heterotrophic organisms. *Proc. Royal Soc. London*, B204, 141-163.

Belkin, S., D.D. Nelson, and H.W. Jannasch. 1986. Symbiotic assimilation of CO_2 in two hydrothermal vent animals, the mussel *Bathymodiolus thermophilus* and the tube worm *Riftia pachyptila. Biol. Bull. Mar. Biol. Lab.*, Woods Hole, 170, 110-121.

Cavanaugh, C.M. 1983. Symbiotic chemoautotrophic bacteria in marine invertebrates from sulfide-rich habitats. *Nature*, 302, 58-61.

Cavanaugh, C.M. 1985. Symbioses of chemoautotrophic bacteria and marine invertebrates from hydrothermal vents and reducing sediments. *Bull. Biol. Soc. Wash.*, 1985(6), 373-388.

Cavanaugh, C.M., S.L. Gardiner, M.L. Jones, H.W. Jannasch, and J.B. Waterbury. 1981. Prokaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila* Jones: possible chemoautotrophic symbionts. *Science*, 213, 340-342.

Cavanaugh, C.M., P.R. Levering, J.S. Maki, R. Mitchell, and M.E. Lidstrom. 1987. Symbiosis of methanotrophic bacteria and deep-sea mussels. *Nature*, 325, 346-348.

Childress, J.J., C.R. Fisher, J.M. Brooks, M.C. Kennicutt II, R.R. Bidigare, and A.E. Anderson. 1986. A methanotrophic marine molluscan (Bivalvia: Mytilidae) symbiosis: mussels fueled by gas. *Science*, 233, 1306-1308.

Comita, P.B., R.B. Gargosian, and P.M. Williams. 1984. Suspended particulate organic material from hydrothermal vent water at 21. *Nature*, 307, 450-453.

Endow, K. and S. Ohta. 1989. The symbiotic relationship between bacteria and a mesogastropod snail, *Alviniconcha hessleri*, collected from hydrothermal vents of the Mariana Back-Arc Basin. *Bull. Jap. Soc. Micro. Ecol.*, 3, 73-82.

Felbeck, H. 1981. Chemoautotrophic potential of the hydrothermal vent tube worm, *Riftia pachyptila* Jones (Vestimentifera). *Science*, 209, 336-338.

Felbeck, H., J.J. Childress, and G.N. Somero. 1981. Calvin-

Benson cycle and sulfide oxidation enzymes in animals from sulfide-rich habitats. *Nature*, 293, 291-293.

Felbeck, H., J.J. Childress, and G.N. Somero. 1983. Biochemical interactions between molluscs and their algal and bacterial symbionts. p. 331-358. In: *Environmental biochemistry and Physiology*, ed. by P.W. Hochachka. The Mollusca, Vol. 2. Academic Press, NY.

Fiala-Medioni, A. 1984. Mise en evidence par microscopie electronique a transmission de l'abondance de bacteries symbiotique dans la branchie de mollusques bivalves de sources hydrothermales profondes. *Comptes Rendu hebd. Seanc. Acad. Sci.*, 298, 487-492.

Fiala-Medioni, A., A.-M. Alayse, and G. Cachet. 1986. Evidence of in situ uptake and incorporation of bicarbonate and amino acids by the hydrothermal vent mussel. *J. Exp. Mar. Biol. Ecol.*, 96, 191-198.

Fisher, C.R., J.J. Childress, A.J. Arp, J.M. Brooks, D. Distel, J.A. Favuzzi, H. Felbeck, R. Hessler, K.S. Johnson, M.C. Kennicutt II, S.A. Macko, A. Newton, M.A. Powell, G.N. Somero, and T. Soto. 1988. Microhabitat variation in the hydrothermal vent mussel, *Bathymodiolus thermophilus*, at Rose Garden vent on the Galapagos Rift. *Deep Sea Res.*, 35, 1769-1791.

Giere, O. and C. Langheld. 1987. Structural organization transfer and biological fate of endosymbiotic bacteria in gutless oligochaetes. *Mar. Biol.*, 93, 641-650.

Hashimoto, J., K. Fujikura, and H. Hotta. 1990. Observations of deep sea biological communities at the Minami-Ensei Knoll. *JAMSTEC Deep. Res.*, 6, 167-180.

Hessler, R.R. and W.M.Jr. Smithey. 1983. The distribution and community structure of megafauna at the Galapagos Rift hydrothermal vents. p. 735-770. In: *Hydrothermal Process at Seafloor Spreading Centers*, eds. by P.A. Rona, K. Bostrom, L. Laubier, and K.L. Jr. smith. NATO Conference Series IV, Plenum Press, New York.

Higgins, I.J., D.J. Best, R.C. Hammond, and D. Scott. 1981. Methane-oxidizing microorganisms. *Micro. Rev.*, 45, 556-590.

Jannasch, H.W. and C.O. Wirsen. 1979. Chemosynthetic primary production at east-Pacific seafloor spreading center. *Biosciences*, 29, 592-598.

Kennicutt, II, M.C., J.M. Brooks, R.R. Bidigare, R.R. Fay, T.L. Wade, and T.J. Mcdonald. 1985. Vent-type taxa in a hydrocarbon seep region on the Louisiana slope. *Nature*, 317, 351-353.

Laubier, L. and D. Desbruyeres. 1984. Les oasis du fond des oceans. *La Recherche*, 15, 1506-1517.

Le Pennec, M. and A. Hily. 1984. Anatomie, structure et ultrastructure de la branchie d'un Mytilidae des sites hydrothermaux du Pacifique Oriental. *Oceanologica Acta*, 7, 517-523.

Le Pennec, M. and D. Prieur. 1984. Observations sur la nutrition d'un Mytilidae d'un site hydrothermal actif de la dorsale du Pacifique oriental. *C. R. Acad. Sci. Paris, Ser. III*, 298, 493-498.

Paull, C.K., B. Hecker, R. Commeau, R.P. Freeman-Lynde, C. Neumann, W.P. Corso, S. Golubic, J.E. Hook, E. Sikes, and J. Curray. 1984. Biological communities at the Florida escarpment resemble hydrothermal vent taxa. *Science*, 226, 965-967.

Rau, G.H. and J.I. Hedges. 1979. Carbon-13 depletion in a hydrothermal vent mussel: suggestion of a chemosynthetic food source. *Science*, 203, 648-649.

Schweimanns, M. and H. Felbeck. 1985. Significance of the occurrence of chemoautotrophic endosymbionts in lucinid clams from Bermuda. *Mar. Ecol. Prog. Ser.*, 24, 113-120.

Smith, D.C. 1979. From extracellular to intracellular: the establishment of a symbiosis. *Proc. Royal Soc. London*, B204, 115-130.

Smith, K.L.Jr. 1985. Deep sea hydrothermal vent mussels: nutrition stage and distribution at the Galapagos Rift. *Ecology*, 66, 1067-1080.

Southward, E.C. 1986. Gill symbionts in thyasirids and other bivalve molluscs. *J. Mar. Biol. Assoc.*, 66, 889-914.

Stein, J.L. 1984. Subtidal gastropods consume sulfur-oxidizing bacteria: evidence from coastal hydrothermal vents. *Science*, 223, 696-698.

Stein, J.L., S.C. Cary, R.R. Hessler, S. Ohta, R.D. Vetter, J.J. Childress, and H. Felbeck. 1988. Chemoautotrophic symbiosis in a hydrothermal vent gastropod. *Biol. Bull.*, 174, 373-378.

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