Reviews

MARINE BIOTECHNOLOGY

Bacterial Endosymbioses of Gutless Tube-Dwelling Worms in Nonhydrothermal Vent Habitats

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Abstract

Gutless tube-dwelling worms of pogonophorans (also known as frenulates) and vestimentiferans depend on primary production of endosymbiotic bacteria. The endosymbionts include thiotrophs that oxidize sulfur for autotrophic production and methanotrophs that oxidize and assimilate methane. Although most of the pogonophoran and vestimentiferan tube worms possess single thiotrophic 16S rRNA genes (16S rDNA) related to γ-proteobacteria, some pogonohorans are known to bear single methanotroph species or even dual symbionts of thiotrophs and methanotrophs. The vestimentiferan *Lamellibrachia* sp. L1 shows symbiotic 16S rDNA sequences of α-, β-, γ-, and ϵ-proteobacteria, varying among specimens, with RuBisCO form II gene (*cbbM*) sequences related to β-proteobacteria. An unidentified pogonophoran from the world's deepest cold seep, 7326-m deep in the Japan Trench, hosts a symbiotic thiotroph based on 16S rDNA with the RuBisCO form I gene (*cbbL*). In contrast, a shallow-water pogonophoran (*Oligobrachia mashikoi*) in coastal Japan Sea has a methanotrophic 16S rDNA and thiotrophic *cbbL*, which may suggest the feature of type X methanotrophs. These observations demonstrate that pogonophoran and vestimentiferan worms have higher plasticity in bacterial symbioses than previously suspected.

Key words: Vestimentifera — pogonophora — endosymbiont — thiotroph — methanotroph

Introduction

Endosymbioses by autotrophic sulfur-oxidizing bacteria (thiotrophs) or methane-oxidizing bacteria (methanotrophs) occur in more than 200 marine invertebrate species that represent 5 or more phyla, depending on phylum classifications (Fisher, 1990; Cavanaugh, 1994). It has been a common consensus that most host animals harbor a single thiotrophic or methanotrophic species. Thiotrophic symbionts carry out chemolithoautotrophic organic production via the Calvin-Benson cycle in which energy for CO_2 fixation by the enzyme RuBisCO (EC 4.1.1.39) derives from sulfide oxidation (Fisher, 1990). Generally RuBisCO has 2 forms, namely I and II. It is hypothesized that the common ancestor of RuBisCOs was similar to form II, as this form is more adaptive to high CO_2 concentration, a condition presumed to have been present for the primitive Earth (Jordan and Ogren, 1981). In contrast, form I is believed to have evolved in response to the decline of CO_2 and emergence of oxygen as the Earth's atmosphere changed (McFadden et al., 1986). The symbiotic thiotrophs are mostly ascribed to γ-proteobacteria, with a few exceptions in α-proteobacteria (Elsaied and Naganuma, 2001; Elsaied et al., 2002). In contrast, methanotrophic symbionts assimilate carbon derived not from CO_2 but from methane, and oxidize part of methane to gain energy for metabolism (Fisher, 1990). Among the 2 major types of methane-oxidizing bacteria belonging to γ-proteobacteria, only the type I group has been found in symbioses (Fisher, 1990; Cavanaugh, 1994). In neither thiotrophic nor methanotrophic symbioses have the organic materials transferred from symbionts to hosts been characterized.

The most studied hosts and symbionts are the gutless tube-dwelling worms of vestimentifera (tube worms) and pogonophora (beard worms). The worms have been reported from a number of hydrothermal vents (Fisher, 1990) and from cold seeps associated

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with hydrocarbon reservoirs, subduction zone accretionary prisms, landslides (McMullin et al., 2003), as well as whale carcasses (e.g., Feldman et al., 1998). The vestimentiferan and pogonophoran tube worms, together with *Sclerolinum brattstromi* (e.g., Halanych et al., 2001) and the bone-eating worms *Osedex rubiplumes* and *Os. frankpressi* (Rouse et al., 2004), compose the family Siboglinidae within the class Polychaeta under the phylum Annelida (Winnepenninckx et al., 1995; McHugh, 1997; Rouse and Fauchald, 1997; McMullin et al., 2003). However, their convergent and homoplastic characters related to oligomerous deuterostomes are still argued (Salivan-Plawen, 2000). These worms share the same unique strategy for life: i.e., lack of digestive tract and dependence on endosymbionts for nutrition (Fisher, 1990), although the endosymbionts of the *Osedex* worms are heterotrophic (Rouse et al., 2004). Therefore the vestimentiferan and pogonophoran worms are referred collectively to "tube worms" in this mini-review.

Tube Worms in Nonhydrothermal-Vent Habitats

Siboglinid evolution is likely associated with sulfide levels of the habitats ranging from soft sediments and methane seeps to hydrothermal vents (Schulze and Halanych, 2003). Vestimentiferans are generally known in high-sulfide vents and seeps (Fisher, 1990; Lutz and Kennish, 1993: McMullin et al., 2003), and associated with whale carcasses (Feldman et al., 1997; Di Meo et al., 2000; Smith and Baco, 2003; Rouse et al., 2004) and shipwrecks (Dando et al., 1992; Williams et al., 1993). The northeastern Pacific vestimentiferans, *Escarpia spicata* and *Lamellibrachia barhami*, show opportunistic occurrence in vent and seep habitats and even on a decaying whale carcass (Black et al., 1997, 1998; Feldman et al., 1998). This opportunism may be explained by low geographic barriers between vent and seep habitats, and similar opportunism should be found for the tube worms of the western Pacific, which has similar geologic and geographic settings. In contrast, pogonophoran tube worms have been mostly reported from low-sulfide habitats such as a Loihi Seamount hydrothermal vent and cold seeps (Flügel and Langhoff, 1983; Schmaljohann and Flügel, 1987; Dando et al., 1994; Black et al., 1997), fjord (Webb, 1963; A.J. Southward et al., 1981), landslide (Mayer et al., 1988), other shallow and deep muddy sediments (e.g., Ivanov, 1963; E.C. Southward, 1971, 1972; Imajima, 1973; A.J. Southward et al., 1981), and from a mud volcano off the Norwegian coast (Pimenov et al., 1999). The pogonophorans are likely to have wider distribution in nonhydrothermal-vent habitats than expected. Tube worms in seeps and muddy sediments have also greater longevity (approx. 200 years or longer; Fisher et al., 1997; Bergquist et al., 2000; Cordes et al., 2003, 2005) than individual hydrothermal vents (approx. 100 years; MacDonald et al., 1980; Killingley et al., 1982; Lalou and Brichet, 1982; Delaney et al., 1998), where focused fluxes of heat and chemicals sustain much larger masses of tube worms. Compared with high fluxes at hot vents, cold seeps and muddy sediments provide only diffusive and slow fluxes, and the tube worms need to develop a "life in the slow lane" (Fisher et al., 1997). Mud volcanoes may supply high but pulsed fluxes of hydrocarbons (MacDonald et al., 2000). Whale carcasses are also ephemeral and able to supply only limited amounts of methane and sulfide (Smith and Baco, 2003).

The slow but long life of the seep tube worms may be suitable for dissecting the process by which occasional bacterial intruders are selected for establishing functional endosymbioses. Bacterial endosymbionts are not heritable in a vent vestimentiferan (Cary et al., 1993), which is likely the common feature of other vestimentiferan and pogonophoran species. Each generation of the tube worms has to introduce ambient microorganisms in their body and select useful ones as endosymbionts for each individual worm. A speculation is that the rapid-growing vent worms (Lutz et al., 1994) may have a rapid process of symbiont selection, and thus tend to host single symbiont species that may vary by habitat (Feldman et al., 1997; Laue and Nelson, 1997; Di Meo et al., 2000). However, non-vent tube worms living "life in the slow lane" would have enough time to select their symbionts, which may vary widely among habitats, individuals, and even symbiont-bearing cells. Thus complexity and flexibility in the endosymbioses of the tube worms inhabiting seeps and muddy sediment are the focus of in this mini-review, although the speculation is yet to be proved.

Endosymbioses by Multiple Bacterial Species

The view of endosymbioses by single bacterial species has been challenged by multiple bacterial endosymbioses in seep and mud tube worms and other vent and seep hosts (Table 1). Some vent mytilid mussels depend on dual symbioses of thiotrophic and methanotrophic bacteria (Distel et al., 1995; Robinson et al., 1998; Fiala-Medioni et al., 2002). The gutless oligochaete *Olavius algarvensis* has an endosymbiotic coalition of a sulfate-reducing bacterium and a thiotroph (Dubilier et al., 2001). In the coalition, sulfate reducing bacterium supplies sul-

Table 1. Examples of Observed and Suspected Multiple Endosymbioses in Marine Invertebrates

Host invertebrate		*Endosymbionts*	*Note*	*Reference*
Seep pogonophoran	*Siboglinum poseidoni*	Thiotroph and methnotroph	Enzymatic activities	Schmaljohann et al., (1990)
Mud pogonophoran	*Oligobrachia mashikoi*	Suspected thio-/methanotroph	16S rDNA and RuBisCO gene in situ hybridization	Kimura et al., (2003a)
Seep vestimentiferan	*Lamellibrachia* sp.	Unidentified	Microscopic observation	Naganuma et al., (1997a)
Seep vestimentiferan	*Lamellibrachia* sp.	ϵ-Proteobacteria	16S rDNA in situ hybridization	Naganuma et al., (1997b)
Seep vestimentiferan	*Lamellibrachia* sp.	α-Proteobacterium (but 2 RuBisCO genes)	16S rDNA and RuBisCO gene in situ hybridization	Elsaied and Naganuma (2001) Elsaied et al., (2002)
Seep vestimentiferan	*Lamellibrachia* sp.	α-,β-, γ-Proteo.	16S rDNA in situ hybridization	Kimura et al., (2003b)
Vent vestimentiferans	*Ridgea* spp. (larvae)	"Large" and "small"	TEM observation	Southward (1988)
Vent vestimentiferans	*Ridgiea piscesae, R. phaeophiale*	"Large" and "small"	TEM observation	de Burgh et al., (1989)
Vent vestimentiferan	*Riftia pachyptila*	"Variety of bacterial types"	Substrate oxidation (methane and sulfide)	Fisher and Childress (1984)
Gutless oligochaete	*Inanidrilus leukodermatus* *Olavius planus*	"Larger" and "smaller"	Light microscopy and TEM Micro X-ray analysis	Giere and Langheld (1987)
Gutless oligochaete	*Olavius loisae*	α-Proteobacterium γ-Proteobacterium	TEM, 16S rDNA Fluorescence in situ hybridization (FISH)	Dubilier et al., (1999) Krieger et al., (2000)
Gutless oligochaete	*Inanidrilus leukodermatus*	"Larger" and "smaller"	"Larger" contains RuBisCO and sulfur	Dubilier et al., (2001)
Gutless oligochaete	*Olavius algarvensis*	γ-/δ-Proteobacterium	Sulfur-oxidizing and sulfate-reducing bacteria	Fujiwara et al., (2001)
Seep clam	*Maorithyas hadalis*	Two thiotrophic phylotypes	Related to known symbionts and free-living forms	Brooks et al., (1987)
Seep mussel	Unnamed mytilid	Methanotroph (type I) Chemoautotroph (?)	Diagnostic enzyme activities, Single methanotroph suggested by 16S rDNA, however, RuBisCO activity suggested, too.	Fisher et al., (1987) Distel and Cavanaugh (1994)
Vent mussel	Unnamed mytilid	Thiotroph, methanotroph	Type I methanotroph, 16S rDNA, FISH	Distel et al., (1995)
Vent mussel	*Bathymodiolus* sp.	Thiotroph, methanotroph	n-8 Monoen fatty acids, $\delta^{13}C = -24.9 \sim -34.9‰$	Pond et al., (1998)
Vent mussel	*Bathymodiolus puteoserpentis*	Thiotroph, methanotroph	Type I methnotroph, RuBisCO, immunoblotting	Robinson et al., (1998)
Vent mussel	*Bathymodiolus* sp.	Thiotroph, methanotroph	Relative abundance of methanotrophs $6\% \sim 15\%$	Trask and Van Dover (1999)
Vent mussel	*Bathymodiolus azoricus*	Thiotroph, methanotroph	TEM, diagnostic enzymes, $\delta^{13}C$, immunoblotting	Fiala-Medioni et al., (2002)
Vent snail	*Olgaconcha tufari*	Thiotroph, methanotroph	CO_2 uptake and CH_4 oxidation	Gal'chenko et al., (1992)
Shipworm bivalve	*Lyrodus pedicellatus*	*Teredinibacter turnerae, others*	*T. turnerae*, N_2-fixing γ-proteobacterium	Distel et al., (2002)

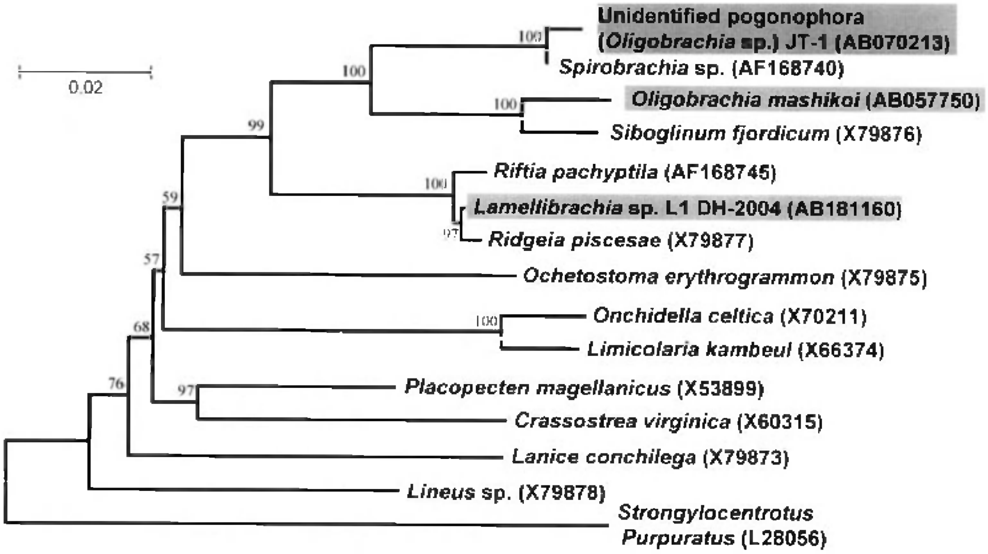

Fig. 1. Phylogenetic tree based on 18S rDNA sequences of the studied pogonophoran and vestimentiferan hosts (shaded) along with representative marine invertebrates. The hosts in this study are *Lamellibrachia* sp. L1, an unidentified pogonophora (tentatively *Oligobrachia* sp. JT-1), and *Oligobrachia mashikoi*. The phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987). The branching pattern of the constructed tree was confirmed by reconstruction using the methods of maximum parsimony and maximum likelihood. Bootstrap values greater than 50 for 1000 replicates are indicated at nodes, and values less than 50 are not reported. Scale bar, 0.02 substitutions per site.

fide for oxidation by the thiotroph, but they should be separated in the host body to ensure anaerobic and aerobic microhabitats, respectively. The microhabitat segregation may be associated with a "physiological gradient" (de Burgh, 1986), which is obscure in *Ola. algarvensis* (Dubilier et al., 2001). The microhabitats may also be separated by a physical barrier, which has not been elucidated.

The undescribed lamellibrachid *Lamellibrachia* sp. L1 (Fig. 1) inhabits a methane seep, 1167 to 1170-m deep, in Sagami Bay, Japan (35°00.1′N, 139°13.6′E; Hashimoto et al., 1989; Masuzawa et al., 1992; Kojima, 2002), and hosts multiple symbionts (Figs. 2 and 3). At least 2 ϵ-proteobacterial cells and 16S rDNA sequences were observed in a specimen (Naganuma et al., 1997a, 1997b). Many species of ϵ-proteobacteria are microaerobes, and the symbiotic ones may occupy a microaerobic niche in an oxygen gradient (Naganuma, 1998, 1999). However, another specimen of *Lamellibrachia* sp. L1 yielded a single 16S rDNA sequence related to the α-proteobacterium *Rhodobacter sulfidophilus* and 2 sequences of the RuBisCO form II gene (*cbbM*) related to that of the β-proteobacterium *Thiobacillus denitrificance* (Elsaied and Naganuma, 2001; Elsaied et al., 2002). The third specimen demonstrated the endosymbiotic localization of α-, β- and γ-proteobacterial 16S rDNA sequences by in situ hybridization (Kimura et al., 2003b). These studies suggest that the symbiotic 16S rDNA sequences in *Lamellibrachia* sp. L1 are highly variable. The varied symbioses should be ascribed not only to individual-to-individual (interindividual) variation but probably also to part-to-part (intraindividual) variation within a single individual due to a physiologic gradient in trophosome (de Burgh, 1986).

It is unclear whether those variable symbioses are already established forms or only transient forms during selection of symbionts acquired from ambient water and sediment. The view of environmental (occasional) acquisition of symbionts is suggested by the occurrence of related (not identical) 16S rDNA sequences in the *Lamellibrachia* trophosome and in the ambient sediment (Kimura et al., 1999). Unequivocal detection of endosymbionts as free-living forms in ambient environment is proved only for the lucinid bivalve *Codaika orbicularis* in shallow-water seagrass bed (Gros et al., 2003). Environmental acquisition of symbionts has been also suggested in gutless oligochaetes (Giere and Langheld, 1987; Giere et al., 1991; Dubilier et al., 1995, 1999, 2001; Krieger et al., 2000) and vent vestimentiferans (Cary et al., 1993; Feldman et al., 1997; Laue and Nelson, 1997). This view is supported by the observations that different species of vestimentiferan worms in the same habitats may bear identical endosymbionts, that the same species of vestimentiferans in different habitats may host different symbionts, and the vestimentiferans and endosymbionts have not necessarily co-evolved (Feldman et al., 1997; Laue and Nelson, 1997; Di Meo et al., 2000), contrary to cospeciation of vesicomyid clams and symbiotic bacteria (Peek et al., 1998).

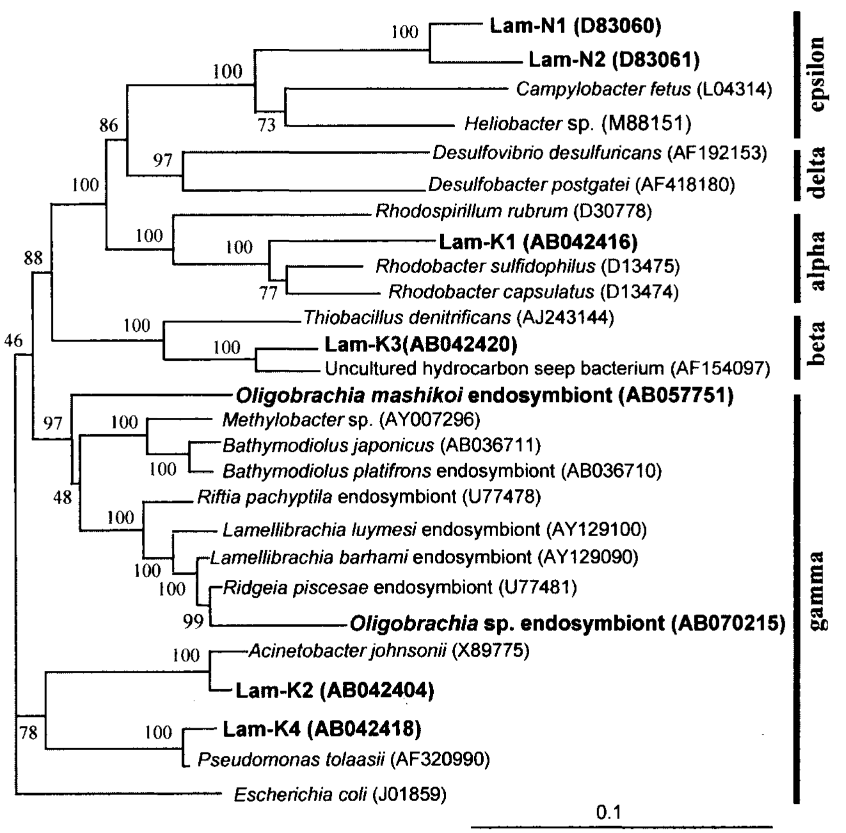

Fig. 2. Phylogenetic tree based on 16S rDNA sequences of representative pogonophoran and vestimentiferan endosymbionts. The endosymbionts in this study are *Lamellibrachia* sp. L1 endosymbionts Lam-N1 and -N2 (TW-1 and -2 of Naganuma et al., 1997b), Lam-K1, -K2, -K3, and -K4 (TW-2, -3, -5, and -6 of Kimura et al., 2003b), an *Oligobrachia* sp. JT-1 endosymbiont (this communication), and an *Oligobrachia mashikoi* endosymbiont (Kimura et al., (2003a). The branching pattern of the constructed tree was confirmed by reconstruction using the methods of maximum parsimony and maximum likelihood. Bootstrap values greater than 50 for 1000 replicates are indicated at nodes. Scale bar, 0.1 substitutions per site.

Possible Dual Thiotrophy/Methanotrophy in Pogonophorans

Previous studies have shown that pogonophoran endosymbionts are thiotrophs or methanotrophs, or both, as demonstrated by transmission electron microscopy, enzymatic activity, and carbon isotope ratio (Dando et al., 1986; A.J. Southward et al., 1986; Spiro et al., 1986; Schmaljohann and Flügel, 1987; Schmaljohann et al., 1990). Combined characterizations of 16S rDNA, the genes encoding RuBisCO forms I and II (*cbbL* and *cbbM*), particulate and soluble methane monooxygenase (*pmoA* and *mmoX*), methanol dehydrogenase (*mxaF*), and a sulfur-oxidizing enzyme (*soxB*) in pogonophoran symbioses have been done in only a few examples. One is the thiotrophic symbiosis of the undescribed pogonophoran worm from the world's deepest cold seep, 7326 m deep, in the Japan Trench (40°02.9′N, 144°16.5′E; Fujikura et al., 1999), which is first reported in this communication. Partial 18S rDNA sequences (accession numbers AB070213 and AB070214) show that the Japan Trench worm is related to the pogonophorans *Oligobrachia mashikoi* (at 87% and 89% similarities, respectively), to *Siboglium fiordicum* (85% and 88%), and to a vestimentiferan, *Ridgeia piscesae* (84% and 88%), as shown in Figure 1. One symbiotic 16S rDNA sequence (AB070215; Fig. 2) is related to a free-living thiotroph from a shallow hydrothermal vent (AF170424) at 90% similarity, and to the thiotrophic symbionts of bivalves such as *Codakia orbicularis* (X84979), *Lucina floridana* (L25707), and *Solemya terraeregina* (U62131) at 88% to 90% similarities.

Partial sequence of the RuBisCO form I gene *cbbL* (620 bp; AB070216) is identified in the Japan Trench worm, and related to those of the γ-proteobacteria *Thiobacillus* sp. (AF038430), *Hydrogenovibrio* sp. (D43622), and the pogonophoran *Oligobrachia mashikoi* endosymbiont (AB057772) at 74% to 76% nucleotide similarities. In contrast, a RuBisCO form II gene, *cbbM*, that occurs in *Lam-*

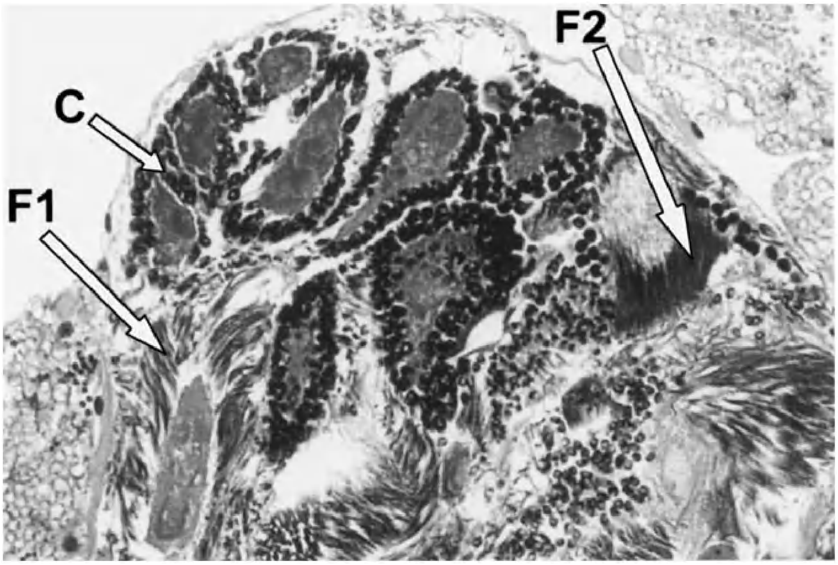

Fig. 3. Light photomicrograph of the cross section of the vestimentiferan *Lamellibrachia* sp. L1 trophosome. Multiple forms of symbiotic microorganisms are stained with eosin-hematoxylin. C indicates coccoid form; and F1 and F2, two different filamentous forms. Photo width, 320 μm.

ellibracha sp. L1 and the genes *pmoA*, *mmoX*, and *mxaF* that are methanotrophic markers (MacDonald et al., 1995; Costello and Lidstrom, 1999) have not been detected.

Pogonophorans such as *Siboglinum atlanticum*, *S. ekmani*, and *S. fiordicum* live in symbiosis with methanotrophic bacteria, contrary to the Japan Trench worm. However, the methanotrophic symbionts may possess RuBisCO, as revealed by culture experiments (Schmaljohann and Flügel, 1987), very low stable carbon isotope ratios (A.J. Southward et al., 1981) and enzymatic activities (Spiro et al., 1986; Schmaljohann et al., 1990). The presence of RuBisCO in methanotrophic symbiosis is not surprising, as type X methanotrophs are capable of autotrophy via the Calvin-Benson cycle (Colby et al., 1979; Hanson and Hanson, 1996).

The pogonophoran *Oligobrachia mashikoi* (18S rDNA, AB057750; Figure 1) is the first described species of the athecanephrid genus *Oligobrachia* in Japanese waters (Imajima, 1973). It is closely related to vestimentiferans in hemoglobin structure (Yuasa et al., 1996; Zal et al., 1998), but differentiated from the phylum Annelid by body wall muscular system (Matsuno and Sasayama, 2002). The studied *Oli. mashikoi* worm lives in shallow muddy sediment

Fig. 4. Tube of the live *Lamellibrachia* sp. L1 embedded in the authigenic carbonate concretion. Photo width, 7 cm.

Table 2. Chemical compositions of Modern and Miocene Carbonate Concretines Containing Worm Tubes

	Miocene carbonate (17.2–14.4 million years ago)		*Off-Hatsushima carbonate colonized by Lamellibrachia sp. L1*
Chemical	*Embedding no fossil tubes*	*Embedding fossil tubes*	
$CaCO_3$	66.6	38.7	39.3
FeO_2	28.4	4.6	5.1
SiO_2	<0.1	28.7	28.0
$MgCO_3$	<0.1	14.8	14.5
Al_2O_3	<0.1	10.0	9.7

and harbors a single γ-proteobacterial symbiont (16S rDNA, AB057751; Figure 2) related to an uncultured bacterium from a hydrocarbon seep and *Methylobacter* sp., and to thiotrophic symbionts of the bivalves *Thyasira flexuosa*, *Codakia costata*, and *Lucina pectinata* (Figure 2; Kimura et al., 2003a). Therefore the *Oli. mashikoi* symbiont has not been determinatively characterized as thiotrophic or methanotrophic. In contrast, RuBisCO form I gene *cbbL* (AB257772) is identified in *Oli. mashikoi* and related to γ-proteobacterial *cbbL* of *Thiobacillus* and *Hydrogenovibrio* species (Kimura et al., 2003a). These 16S rRNA and RuBisCO form I genes are located in the *Oli. mashikoi* trophosome by in situ hybridization (Kimura et al., 2003a). It should be noted that the RuBisCO form II gene (*cbbM*), methanotrophic marker genes (*pmoA*, *mmoX*, and *moxF*) and a thiotrophic marker gene (*soxB*) have not been amplified by polymerase chain reaction from the *Oli. mashikoi* trophosome (Kimura et al., 2003a. These data neither specify thiotrophic or methanotrophic nature of the symbiont, nor rule out the possibility of dual thiotrophy and methanotrophy known in type X methanotrophs (Colby et al., 1979; Hanson and Hanson, 1996). Symbiosis by a type X methanotroph has been suspected in pogonophorans (E.C. Southward, 1982) and vent bivalves (Robinson et al., 1998; Fiala-Medioni et al., 2002).

Strategies for Sulfide Exploitation

Thiotrophy of the tube worms symbioses is predominantly seen in hydrothermal vent habitats with sulfide concentrations as high as approx. 111 mM (e.g., Shanks et al., 1995). Vestimentiferans take up sulfide and dissolved oxygen from the uppermost (anterior) "plume" standing into ambient water as high as tens of centimeters to a few meters. In contrast, sulfide concentration in the bottom waters overlying cold seeps and muddy sediments varies from 20 mM (Girguis et al., 2002) to 1 μM (Julian et al., 1999). Sulfide is present only in interstitial water and virtually absent in the overlying water of the Gulf of Mexico seeps dominated by *Lamellibrachia* cf. *luymesi* (Freytag et al., 2001) and the so-called off-Hatsushima seep inhabited by *Lamellibrachia* sp. L1 (Gamo et al., 1988; Masuzawa et al., 1992; Hashimoto et al., 1995). In the sulfide-depleted water, *L.* cf. *luymesi* is known to take up sulfide from the posterior "root"; however, the relative importance of sulfide uptake via root versus plume is still unclear.

Pogonophoran worms may take up sulfide from the deep buried posterior part of the body, and the endosymbionts are mainly found in the posterior part of the trophosome (E.C. Southward, 1982). Similarly, seep vestimentiferans, particularly lamellibrachids, take up sulfide in the interstitial water from their posterior part, or root, extended deeply into sediment (MacDonald and Fisher, 1996; Julian et al., 1999; Freytag et al., 2001). The root growth of lamellibrachids is similar to the "sulfide mining" observed in symbiotic thyasirid clams using the superextensile foot as the part of sulfide uptake (Dufour and Felbeck, 2003). The lamellibrachid "root hypothesis" (Julian et al., 1999; Freytag et al., 2001) is intriguing; however, lamellibrachids may not promptly reposition their roots in the carbonate-cemented sediment to the upward-downward shift of the sulfide formation zone, namely, the sulfate-methane interface (SMI; e.g., DeLong, 2000). Upward shift of SMI may lower sulfide uptake, because upper parts of worm tubes are thicker and thus less permeable to sulfide (Julian et al., 1999; Freytag et al., 2001), and the sulfide-permeable posterior part should grow upward to the shallower SMI. Downward shift of SMI is associated with lowered seepage and would force the roots to grow farther downward. Tube worms may thus keep up with SMI shifts by upward and downward growth of the roots within a lifetime, and worm tubes with several bends in their sub-seafloor parts are often observed. In contrast, the vesicomyid clam *Calyptogena soyoae* adjusts the depth of foot extension according to the depth of SMI (Hashimoto et al., 1995).

Tubes of *Lamellibrachia* sp. L1 are buried fast in carbonate concretion in the off-Hatsushima seep (Fig. 4), and tubes grow upward to complement the fast burial. As the lamellibrachid root grows downward, it penetrates the sulfide-forming SMI and reaches the sub-SMI zone with relatively high methane but low-sulfide concentrations (Masuzawa et al., 1992). The depth of SMI is often recorded on the tubes of *Lamellibrachia* sp. L1 as shown by the blackish zone oxidized via sulfate reduction (i.e., "burnt" by sulfate), and the roots often extend to tens of centimeters below the burnt zone (unpublished results).

The root hypothesis implies enhanced regeneration of sulfide in the seep sediment in which interstitial sulfate has been already reduced to sulfide. Extra sulfate comes from overlying seawater via the worm's body fluid circulation and from endosymbiotic sulfide oxidation. The root hypothesis thus requires the coalition of endosymbiotic thiotrophs and epibiotic sulfate-reducing bacteria in sediment, which contrasts the dual endosymbiotic coalition of a thiotroph and a sulfate-reducing bacterium in *Olavius algarvensis* (Dubilier et al., 2001). An other possibility for the solution to the sulfide deficiency problem is occasional dependence on thiotrophy and methanotrophy as mentioned above.

Colonization and Fossilization of Seep Tube Worms

The off-Hatsushima seep is also colonized by the endobenthic vesicomyid clam *Calyptogena soyoae* (Hashimoto et al., 1989). Living *Calyptogena* clams move in the soft muddy sediment to exploit sulfide and methane (Hashimoto et al., 1995), and thus rarely occur in the carbonate concretions that often exist beneath the superficial sediment or occasionally exposed on the seafloor (Ohta, 1990). In contrast, the *Lamellibrachia* worm anchors its posterior part to the carbonate concretions, and thus shows habitat segregation with the *Calyptogena* clams.

The seep carbonates are mostly authigenic calcite derived from oxidation of methane in the seep fluids (Hattori et al., 1994). The off-Hatsushima seep represents modern seeps in the subduction zone of the Philippine Sea Plate against the North American Plate, and its Miocene counterpart (17.2–14.4 Mya) has been identified in the Miura Peninsula, 40 km east of the modern seep (Naganuma et al., 1995). Chemical compositions of the modern and Miocene carbonate concretions that contain worm tubes are closely similar and regarded as high-magnesium carbonate (Table 2). In contrast, the Miocene carbonate without worm fossils is clearly distinguished, even though it occurs next to the tube-containing carbonate.

The high magnesium content in the tube-containing Miocene and modern seep carbonates may be closely coupled with the colonization of lamellibrachid tube worms. It is known that a high-magnesium carbonate, or possibly dolomite, is formed as a result of biological sulfate reduction (Vasoncelos et al., 1995). X-ray microanalysis of the Miocene worm tubes showed co-accumulation of sulfur and iron, indicative of iron sulfide (probably pyrite), inside of the tubes, where soft body was positioned. Iron may have derived from the hemoglobin in the worm's blood, yielding iron-sulfur minerals such as iron sulfide (Naganuma et al., 1995). Iron sulfide may further react with sulfide to yield pyrite under highly sulfidic conditions, for example, tube worm decay after death. The pyrite is also observed in Cretaceous seep tube fossils (Beauchamp et al., 1989).

The Miocene worm tubes were embedded in calcite, and the co-occurrence of calcite and pyrite is an indicator of hypoxic oxygen level as low as 0.3 to 1.0 ml O_2/L (Brett and Baird, 1986). Tube worms recharge the hypoxic to anoxic sediment with sulfate according to the root hypothesis. As a result sulfate would be possibly precipitated on the inner or outer surface of the worm tubes as calcium sulfate, which is the dominant form of sulfate in the deep-sea reducing habitats. However, x-ray microanalysis on the Miocene and modern worm tubes showed no co-accumulation of sulfur and calcium (Naganuma et al., 1995, 1996), which can not be explained solely by the root hypothesis.

Biotechnological Perspectives

Tube worms live in chitinous tubes (Gaill et al., 1992) secreted in the expense of symbiont-fixed carbon (Felbeck and Jarchow, 1998; Bright et al., 2000), and uses for chitin derivatives such as chitosan in novel drug delivery systems, wound healing, and anticoagulation have been developed (Hirano, 1996). Animals possess 2 forms of carbamylphosphate synthetase connected separately to the syntheses of arginine and pyrimidine nucleotides; however, the vent tube worm *Riftia pachyptila* lacks enzymatic activities involved in the pyrimidine nucleotide synthesis (Simon et al., 2000; Minic et al., 2002). This feature, which may be shared by nonhydrothermal-vent tube worms, suggests that host enzymatic activities are repressed by a symbiont and thus may be applied to develop a novel way to inhibit enzyme activity or gene expression via bacterial symbioses. This aspect of host-symbiont interaction also has relevance to bacterial quorum-sensing autoinduction and signal transduction, and the signal kinase and signal regulator gene homologues are isolated from the *R. pachyptila* symbiont fosmid library (Hughes et al., 1997).

Conclusion

Tube worms, such as vestimentiferans and pogonophorans, inhabiting seeps and muddy sediments face a sulfide deficiency problem and thus develop different endosymbioses from the thiotrophy-based symbioses of the vent vestimentiferans. Some seep tube worms depend on symbiotic methanotrophy or dual thiotrophy/methanotrophy. The vestimentifera *Lamellibrachia* sp. L1 has multiple heterogeneous endosymbionts, which may reflect different stages in acquisition and selection of candidate microorganisms for functional endosymbioses. Sulfide exploitation by seep tube worms has geochemical relevance to sulfate regeneration in anoxic sediment.

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