Microbial Symbiosis: Patterns of Diversity in the Marine Environment[1]

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SYNOPSIS. Symbiotic associations expand both the diversity of potential ecological niches and metabolic capabilities of the host-symbiont combinations. Symbioses can also be considered to have evolutionary potential in that the partnership can result in a "new organism." Associations between chemoautotrophic bacteria and marine invertebrates, discovered only 10 years ago, are now found widely in nature, in habitats ranging from deep-sea hydrothermal vents to coastal sediments. Here I review chemoautotroph-invertebrate associations and discuss the benefits inferred for both partners with regard to the diversity of these symbioses in nature.

INTRODUCTION

The term "biodiversity" typically evokes an image of the wide variety of *individual* species of plants, animals, and microorganisms. Symbioses, in which two or more species live together, bring a new level of complexity to the study of biodiversity. Many symbiotic associations are known, notably between plants, animals, or protists on the one hand and microorganisms on the other (Smith and Douglas, 1987). For example, lichens are composed of fungal and algal partners; many corals and sea anemones harbor intracellular photosynthetic algae, and leguminous plants include nitrogen fixing bacteria in root nodules. Associations between chemosynthetic bacteria and marine invertebrates, only discovered in 1981, are now found widely in nature. Thus symbioses charge us to think about biodiversity on a different level.

Symbiotic associations between two species often result in evolutionary novelty, enabling one partner to acquire the traits of the other (Margulis and Fester, 1991). This may result in increased metabolic diversity of plants and animals. For example, cows and termites "acquire" the ability to digest cellulose via their gut microflora and invertebrates "become" photosynthetic via symbiosis with algae. The study of symbiosis has taken on increased importance with the hypothesis that the eucaryotic cell may have evolved from a microbial symbiosis with mitochondria originating from aerobic bacteria and plastids from cyanobacterial ancestors (Margulis, 1981; Gray, 1989). Studies of modern symbioses, therefore, broaden our recognition of biodiversity and suggest much about how new biological entities might have arisen.

The discovery of widespread symbioses between marine invertebrates and chemosynthetic procaryotes in the last decade (Cavanaugh, 1985; Fisher, 1990) emphasizes the prominence of animal-bacteria associations and the need to expand our search for associations involving microorganisms. Although it took the exotic environment of hydrothermal vents to catalyze the initial discovery of this symbiosis, subsequent examination of invertebrates from other habitats where sulfide and oxygen both occur has revealed that similar symbioses exist even in well described invertebrate species from more accessible habitats such as coastal sediments. These symbioses had been overlooked presumably because zoologists are accustomed to studying animals and microbiologists are accustomed to studying bacteria. This suggests more interdisciplinary approaches are needed to study the diverse array of organismic biology. As an example of the biodiversity of symbiotic

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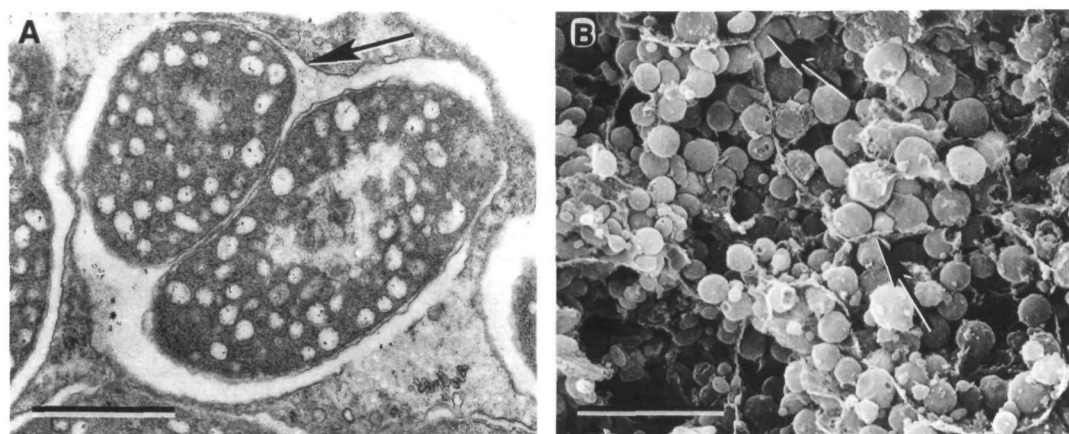
FIG. 1. *Riftia pachyptila*. A. Transmission electron micrograph of trophosome section showing Gram-negative bacterial symbionts contained within a vacuole bound by a membrane (arrow). B. Scanning electron micrograph showing symbionts within trophosome; arrows indicate likely host cell membranes. Scale bars: A, 1 μm; B, 10 μm. Reprinted with permission from *Science and Nature* (Cavanaugh *et al.*, 1981; Cavanaugh, 1983).

associations, I describe here the range of invertebrate species found in symbiosis with sulfur-oxidizing chemoautotrophs. The evolutionary relationship of the symbionts to each other and to free-living bacteria are described, and the inferred benefits to both partners are discussed.

CHEMOSYNTHETIC SYMBIOSES

A symbiotic association between sulfur-oxidizing chemoautotrophic bacteria and marine invertebrates was first proposed for the deep-sea hydrothermal vent tubeworm, *Riftia pachyptila* (Cavanaugh *et al.*, 1981; Felbeck, 1981). These giant tubeworms, up to 2 m in length, were discovered at 2,500 m depth living amidst a flourishing benthic community at warm water vents (up to ~22°C) associated with mid-ocean ridge spreading centers. Chemosynthetic production of bacterial biomass using geothermally produced chemicals such as hydrogen sulfide emitted in the vent fluid and oxygen from ambient seawater has been proposed as the base of the food chain of these communities. However the food source for this new species of tubeworm presented an enigma. *R. pachyptila* are unusual in that, like the related smaller benthic tubeworms of the phylum Pogonophora, they completely lack a mouth and a gut!

A mutualistic symbiosis with sulfur-oxidizing chemoautotrophic bacteria was pro-

posed when crystals of elemental sulfur (an intermediate product in the oxidation of sulfide) were detected in the tubeworm trophosome, a brown spongy tissue which fills the bulk of their coelomic cavity (Cavanaugh *et al.*, 1981). Such bacteria are capable of deriving their energy from the oxidation of reduced inorganic sulfur compounds such as hydrogen sulfide, thiosulfate, or elemental sulfur and their carbon from CO_2, a process called chemosynthesis. Potential benefits of chemoautotroph-invertebrate associations for the host include an internal source of organic compounds produced by their symbionts. The host may also benefit from symbiont detoxification of sulfide, a potent metabolic inhibitor, via oxidation to non-toxic compounds such as elemental sulfur or sulfate. Potential benefits for the microbial symbiont include simultaneous access provided by the host to both sulfide and oxygen from the environment.

Indeed, examination of the trophosome tissue of *R. pachyptila* with both light and electron microscopy reveals the presence of numerous procaryotic cells, up to 10^9 per gram wet weight of tissue, which occur intracellularly (Fig. 1) (Cavanaugh *et al.*, 1981). The co-occurrence of enzyme activities associated with sulfur oxidation and activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (Felbeck, 1981), the CO_2-fixing enzyme diagnostic of
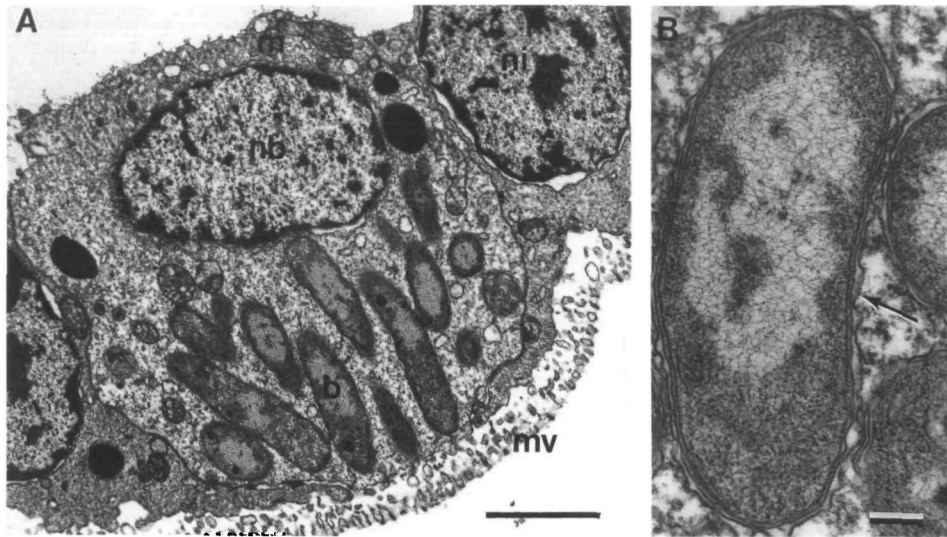
FIG. 2. *Solemya velum.* A. Transmission electron micrograph, transverse section of gill filament, showing rod-shaped bacteria within gill bacteriocyte ana intercalary cells lacking symbionts; b: bacteria; mv: microvilli; nb: nucleus of bacteriocyte; ni: nucleus of intercalary cell. B. Same, higher magnification, transverse section of rod-shaped bacterium, showing cell ultrastructure typical of Gram-negative bacteria and peribacterial membrane (arrows). Scale bars, A: 3 μm; B: 0.2 μm. Reprinted with permission from *Biol. Soc. Wash. Bull.* (Cavanaugh, 1985).

the Calvin cycle known only to occur in autotrophs, suggests that the bacteria observed in the trophosome are sulfur-oxidizing chemoautotrophs. Stable carbon isotope data indicate that these worms do depend on bacterial carbon as the main source of their nutrition (Rau, 1981). The trophosome is highly vascularized and the

TABLE 1. *Marine invertebrate—chemoautotroph symbioses.**

Phylum	Symbiont-containing tissue	Location	Habitat
Vestimentifera	trophosome	intracellular	deep-sea hot vents, cold seeps
Pogonophora	trophosome	intracellular	shelf and slope reducing sediments
Mollusca			
Class Bivalvia	gills	intracellular or extracellular	deep-sea hot vents, cold seeps, reducing sediments
Family Solemyidae			
Lucinidae			
Vesicomyidae			
Mytilidae			
Mactridae			
Class Gastropoda	gills	intracellular	deep-sea hot vents
Annelida			
Class Oligochaeta			
Subfamily Phallodrilinae	subcuticular space	extracellular	coralline sands
Nematoda			
Subfamily Stilbonematinae	cuticle	epicuticular	reducing sediments

* Symbioses with sulfur-oxidizing chemoautotrophic bacteria have been described for certain species in each of the invertebrate groups listed. Characterization of the symbionts is based on microscopical observations of Gram-negative bacteria and enzymatic and physiological assays diagnostic of sulfur oxidation and autotrophy. See references listed in reviews (Cavanaugh, 1985; Fisher, 1990; Fiala-Médioni and Felbeck, 1990) and cited in text.

blood of the worm contains a unique extracellular hemoglobin which is capable of binding both oxygen and sulfide (Arp et al., 1987), perfect for transporting these compounds from the mixing ambient seawater and hydrothermal fluids to the symbionts within its inner tissues.

While hydrothermal vents are a spectacular example of an environment supporting invertebrate-bacteria symbioses, the conditions necessary to support chemosynthesis also exist in other environments. For example, in coastal marine sediments up to 90% of the total decomposition of organic matter may occur via bacterial sulfate reduction, resulting in high porewater sulfide concentrations (Howarth and Teal, 1980; Jørgensen, 1982). Thus similar symbioses could exist for invertebrates living in marine sediments where continuous supplies of both sulfide (from biological sulfate reduction) and oxygen (from overlying seawater) are available (Cavanaugh et al., 1981).

Such a symbiosis between sulfur-oxidizing chemoautotrophs and the Atlantic coast protobranch bivalve, Solemya velum, was demonstrated (Cavanaugh et al., 1981, 1988). Members of the genus Solemya, which had been studied since the 1800s, were poorly understood in terms of their mode of nutrition (Kellogg, 1892; Yonge, 1939). They were presumed to be deposit feeders, but their feeding appendages do not reach outside the shell! Furthermore their digestive tracts are extremely small. Examination of various tissues, using assays for RuBisCO activity and electron microscopy revealed intracellular Gram-negative bacteria, up to 10^9 per gram wet weight, in the gills of S. velum (Fig. 2). While S. velum does have a functional gut, feeding studies indicate that it is only able to ingest small particles such as bacteria, and that the rate of ingestion could only provide a few percent of the estimated daily carbon requirements (Krueger et al., 1992). Studies of sulfide-stimulation of CO_2 fixation, stable isotopes, and biochemical composition indicate that the symbionts play the major role in the nutrition of this species (Cavanaugh, 1985; Conway et al., 1989; Conway and Capuzzo, 1991). A seemingly peculiar habit of these clams, building Y-shaped

tubes in the sediment (Stanley, 1970), now makes sense in light of this symbiosis. The symbionts, which occur in the apical region of the cells closest to the seawater, gain ready access to the substrates necessary for chemosynthesis as the clam pumps water through its mantle cavity (anterior to posterior) from the overlying oxygenated seawater through the upper part of the Y and ventrally from the anaerobic porewater through the lower part of the Y, respectively. Thus, S. velum displays both behavioral and anatomical adaptations as an apparent result of this symbiosis.

Similar symbiotic associations between sulfur-oxidizing chemoautotrophs and marine invertebrates have now been proposed for over 100 species from five different phyla collected from diverse marine habitats in which sulfide and oxygen co-occur (Table 1; see reviews: Cavanaugh, 1985; Fisher, 1990). Since the bacterial symbionts have not yet been isolated in pure culture from any of the animals, the primary evidence describing the nature of these symbioses has been microscopical observations of Gram-negative bacteria associated with the host animal, enzymatic and physiological assays diagnostic of sulfur oxidation and autotrophy, and biochemical studies. With the recent characterization of bacteria living on the surface of nematodes, invertebrate associations with chemoautotrophs have been extended to include both epi- and endosymbioses.

As in R. pachyptila, all other species of vestimentiferans examined to date contain intracellular chemoautotrophic symbionts within their trophosome (Southward et al., 1981). Pogonophorans, when re-examined in the light of the discovery of the Riftia symbiosis, were also shown to have a trophosome full of intracellular symbionts. Pogonophorans live in reducing sediments with their tubes spanning the oxic-anoxic interface, giving them access to both sulfide and oxygen. Like vestimentiferans, they completely lack mouth and gut. While these animals are capable of taking up dissolved organic carbon across their epidermis, stable carbon isotope ratios indicate that the symbionts provide the major source of nutrition (Southward et al., 1981).

Symbioses between molluscs and che-moautotrophs have also appeared in widely different families (Table 1), including a number of bivalves and one species of gastropod collected from hydrothermal vents (see review: Fiala-Médioni and Felbeck, 1990). Bivalve-chemoautotroph symbioses are an important component of a number of different marine environments, ranging from intertidal sediments to the deep-sea hydrothermal vents. Among the bivalves, such symbioses occur for all members examined of the families Solemyidae, Lucinidae, and Vesicomyidae, but only in certain deep-sea hydrothermal vent and cold seep species of the family Mytilidae and one species of the family Mactridae (see review: Fiala-Médioni and Felbeck, 1990). As in *S. velum*, the symbionts occur within the host gills which are enlarged relative to non-symbiotic bivalves, and in most species they are intracellular, within gill epithelial cells. The bivalves exhibit varying degrees of dependence on internal symbionts for nutrition. For example, symbionts serve as the major source of nutrition for members of the genus *Solemya* (Conway *et al.*, 1989; Krueger *et al.*, 1992) which includes the completely mouthless and gutless species, *S. reidi*, whereas in other species, filter feeding has the potential to provide a significant fraction of the host's carbon requirements (Fiala-Médioni and Felbeck, 1990). Apparent host adaptations for the provision of substrates to their symbionts include behavioral traits, *e.g.*, physically spanning anoxic-oxic sediment interfaces with burrows, and biochemical traits, *e.g.*, evolving sulfide-binding pigments in the blood of the vent clam *Calyptogena magnifica* (Arp *et al.*, 1984).

Two species of tubificid oligochaetes, *Inanidrilus leukodermatus* and *Olavius planus*, subfamily Phallodrilinae, have also been demonstrated to live in symbiotic association with sulfur-oxidizing chemoautotrophs (Giere, 1989). In these oligochaetes, the symbionts are extracellular, between epidermal cell extensions beneath the worm's cuticle. As in other mouthless and gutless species, biochemical and stable isotope analyses indicate the symbionts provide an internal source of nutrition to their hosts. Both species are found in highest

abundance at the oxic/anoxic interface, or chemocline, in coralline sands. In experimental cores in the laboratory, these worms move to this interfacial region. Thus these hosts appear to provide their symbionts with the necessary substrates for chemosynthesis by tracking the chemocline.

The occurrence of bacteria on the outside of nematodes of the family Stilbonematinae, a small subfamily of marine free-living nematodes were first reported in 1959 (Weiser, 1959). Recently, enzymatic and biochemical evidence has been presented that these epi-bacteria are sulfur-oxidizing chemoautotrophs (Ott *et. al.*, 1991; Polz *et al.*, 1992). Each member of this nematode family is characterized by a species-specific coat of Gram-negative bacteria, which varies in its morphology and arrangement in either mono- or multilayers for different nematode species. One of the most spectacular examples is shown in Figure 3. Here bacteria cover the surface of the nematode *Eubostrichus cf. parasitiferus* in a very ordered arrangement, attaching at both ends to the worm's cuticle. Studies based on host tissue and symbiont stable carbon isotope values indicate that nematodes in this group appear to derive their nutrition by directly feeding on their symbionts. Like the oligochaetes, the nematodes are found in greatest abundance at the oxic-anoxic interfaces in marine sands, and they track the chemocline to provide their symbionts access to both sulfide and oxygen.

Numerous other reports hint that many more examples of both chemoautotrophic epi- and endosymbioses exist in nature. Filamentous bacteria, resembling members of the genus *Thiothrix*, sulfur-oxidizing bacteria long suspected and recently proven to be autotrophic (Kelly *et al.*, 1992) are noted on the surfaces of the alvinellid polychaetes (Desbruyères *et al.*, 1983) and on the surfaces of the shrimp *Rimicaris exoculata* from hydrothermal vents (Van Dover *et al.*, 1988). While the use of sulfur as an energy source has not yet been demonstrated for these symbionts, RuBisCO activity and CO_2 fixation has been associated with the surface of the shrimp, suggesting these epibionts are autotrophic (Wirsen *et al.*, 1992; Gal'chenko *et al.*, 1989). *Thiothrix*-like organisms have
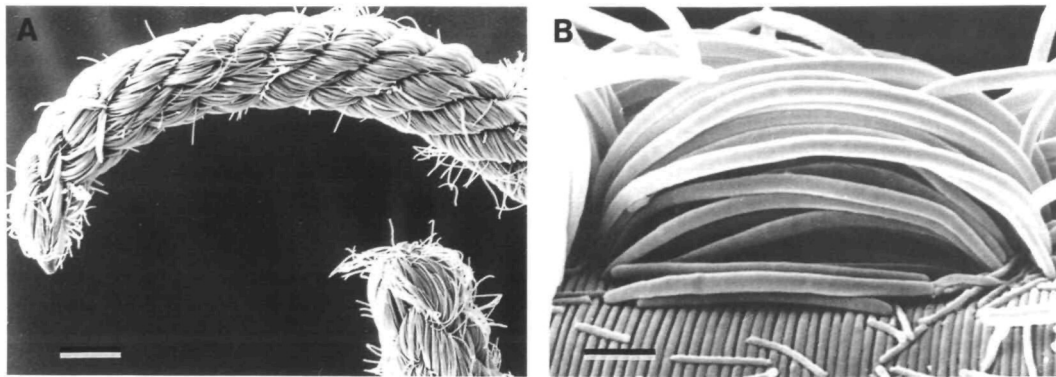
FIG. 3.   *Eubostrichus cf. parasitiferus.* Scanning electron micrographs showing the symbiotic bacteria on the surface of the nematode. A. Anterior (bottom) and posterior (top) end with symbionts arranged in a characteristic helix. B. Higher magnification. Bacteria are attached with both ends to the worm's cuticle. Note the increasing length of the cells from proximal to distal from the worm's surface. Scale bars: A, 20 μm; B, 2 μm. Reprinted with permission from *Microbial Ecology* (Polz *et al.,* 1992).

also been observed attached to the oligochaete *Tubificoides bennedi* on their posterior end which pokes out of anoxic muds (Dubilier, 1986); on the surface of the brackish water priapulid *Halicryptus spinulosus* (Oeschger and Schmaljohann, 1988); and on freshwater mayfly larvae which live at the junction of a sulfur spring and freshwater creek (Larkin *et al.,* 1990). Bacteria on the surface of the sand ciliate *Kentrophorus* have also been suggested to be sulfur-oxidizing chemoautotrophs (Fenchel and Finlay, 1989), as have endosymbionts observed within a gutless nematode and in a gutless turbellarian collected from the oxic-anoxic interface of reducing coastal sediments (Ott *et al.,* 1982). While further characterization is needed to establish whether the bacteria observed associated with these various hosts are capable of autotrophic growth on reduced inorganic sulfur compounds, these observations suggest that symbioses between sulfur-oxidizing chemoautotrophs and eucaryotes may be even more widespread than currently recognized.

SYMBIONT PHYLOGENY

While it is easy to describe the diverse array of animal hosts which form symbioses with chemoautotrophs, characterization of the bacteria, which have not yet been cultured, is more difficult. The bacterial symbionts vary in shape, size, and ultrastructure between different hosts, suggesting that they may be different species. However, bacteria living within cells often are pleomorphic. Fortunately, molecular approaches are now available to begin to determine the phylogenetic relationships of bacteria including unculturable microorganisms (Woese, 1987).

Comparative sequence analysis of 16S rRNA sequences has now been used to determine the phylogenetic relationships of chemoautotrophic symbionts of six different species of bivalves and the vent tubeworm *Riftia pachyptila* (Distel *et al.,* 1988; Eisen *et al.,* 1992) (Fig. 4). These results indicate that the symbionts are species-specific, *i.e.,* individual members of a given invertebrate host have the same symbiont. Although the tested symbionts include species from both hydrothermal vents and reducing sediments, they form one cluster within the Proteobacteria, one of the 11 major groups of eubacteria. *Thiomicrospira* L-12, an isolate from hydrothermal vents, is the closest related free-living species of sulfur bacteria. While the Proteobacteria class includes many species found in association with eucaryotes, including both parasitic and mutualistic symbionts of plants (*e.g.,* agrobacteria and rhizobia) and animals (*e.g.,* enteric organisms and rickettsia) (Stackebrandt *et al.,* 1988), the position of the chemoautotrophic symbionts indicates that they are from an evolutionarily distinct group of bacteria (Distel *et al.,* 1988; Eisen *et al.,* 1992).
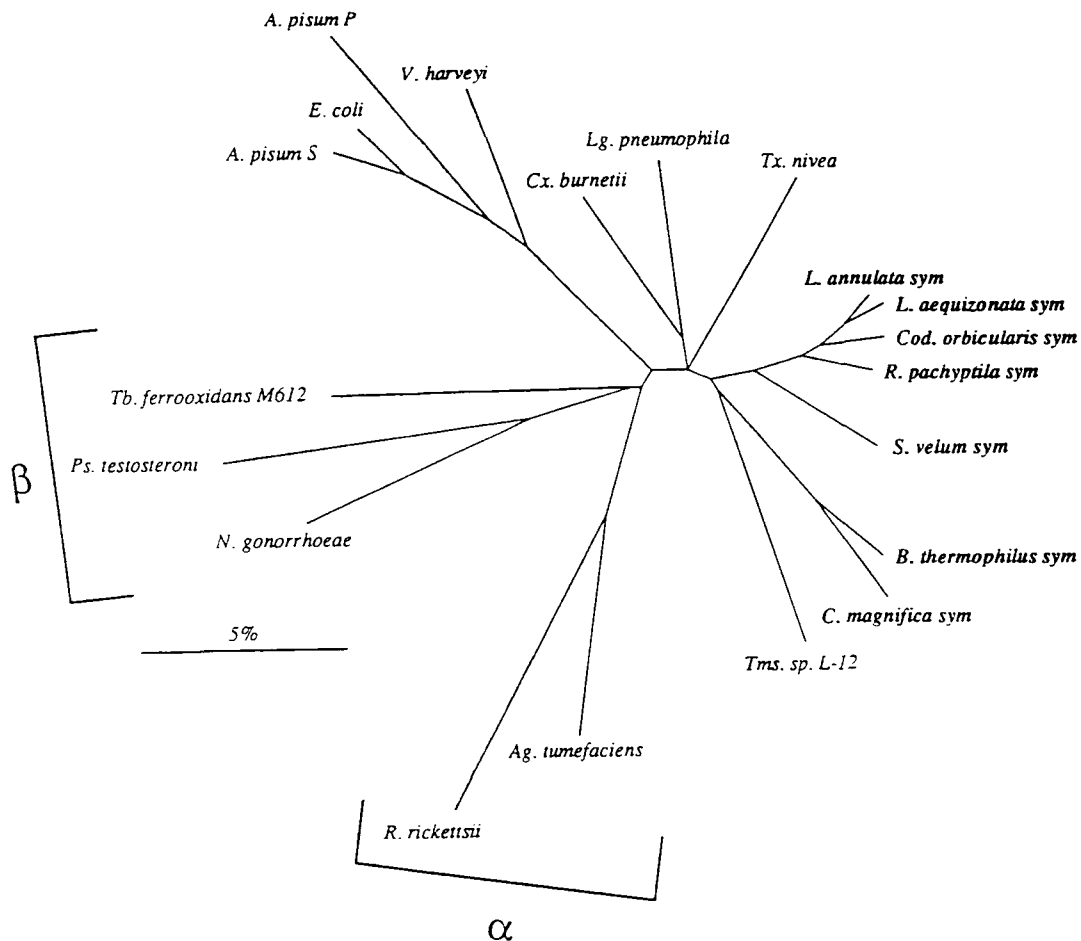
It appears that these symbioses may have

FIG. 4. Unrooted phylogenetic tree, based on evolutionary distances, showing the position of the chemoautotrophic symbionts in relation to that of other Proteobacteria on the basis of 16S rRNA gene sequences. Members of the alpha- (*Rickettsia rickettsii* and *Agrobacterium tumefaciens*) and beta-subclasses (*Thiobacillus ferrooxidans* M612, *Pseudomonas testosteroni*, and *Neisseria gonorrhoeae*) of the Proteobacteria are bracketed; all others are of the gamma subclass. These include chemoautotrophic symbionts (listed in bold type) of bivalves (*Lucinoma aequizonata* sym, *L. annulata* sym, *Codakia orbicularis* sym, *Solemya velum* sym, *Bathymodiolus thermophilus* sym, *Calyptogena magnifica* sym) and the vent tubeworm (*Riftia pachyptila* sym), free-living species (*Thiomicrospira* sp. L-12, *Thiothrix nivea*, *Legionella pneumophila*, *Coxiella burnettii*, *Escherichia coli*, and *Vibrio harveyi*), and aphid symbionts, *Acyrthosiophon pisum* S and *Acyrthosiophon pisum* P. Sym = symbiont. Reprinted (with modifications) from *Journal of Bacteriology* (Eisen *et al.*, 1992).

evolved separately in many host lineages, because the symbiont trees do not appear to parallel the host classifications (Distel *et al.*, 1988; Eisen *et al.*, 1992). For example, the symbionts of the tubeworm *R. pachyptila* occur between two branches of bivalves (Fig. 4), supporting the idea of multiple origins of the symbioses. Further studies of host evolutionary histories are needed so that the phylogenetic relationships of symbionts may be compared with those of their hosts.

ADAPTATIONS TO LIFE AT OXIC-ANOXIC INTERFACES

Although initially proposed as endosymbioses, in the last 10 years the hypothetical stages which would be expected over the course of evolution from two individual species living separately in the same environment to one species living within the cells of another are now observed in current examples of chemosynthetic symbioses. These include: (1) episymbionts living on

the surfaces of animals such as nematodes, (2) endosymbionts which occur within hosts, albeit extracellularly as do subcuticular symbionts of oligochaetes, (3) endosymbionts living intracellularly in specialized cells like the gill symbionts of bivalves or within specialized tissues as seen for the trophosome symbionts of vestimentiferan and pogonophoran tubeworms.

The possible benefits to the partners in chemosynthetic symbioses are of varying importance in these symbioses. The role of the symbionts in the nutrition of their hosts ranges from being the main source for mouthless and gutless species, to being an additional food source for species which have a functional gut and are capable of ingesting particles. The role of symbionts in sulfide detoxification is less apparent. With the exception of the nematodes whose bodies are virtually covered by bacteria, the bacteria in endosymbioses are localized in specific tissues of invertebrates, and therefore other parts of the animal are exposed to sulfide in their environments. Furthermore, other species found in these same habitats lack symbionts, yet are exposed to similar high concentrations of sulfide (cf. Krueger et al., 1992).

In all of these associations, the symbionts appear to benefit from the increased availability of substrates necessary for chemosynthesis, notably sulfide and oxygen, by associating with eucaryotic hosts. Free-living sulfur-oxidizing chemoautotrophs are limited to those narrow zones in which both sulfide and oxygen coexist. Sulfide is produced during organic decomposition by sulfate-reducing bacteria in the absence of oxygen or by geothermal reduction of seawater sulfate emitted in hydrothermal fluids (Postgate and Kelly, 1982). Thus, sulfide is mainly produced anaerobically and must diffuse to oxic zones. Furthermore, sulfide is short-lived in oxic environments as a result of its spontaneous oxidation in the presence of oxygen (Chen and Morris, 1972). Thus the zone where sulfide and oxygen co-occur is not only limited in space but also fluctuates in response to physical and biological processes. In sediments this zone may vary vertically with the amount of organic matter input, or diurnally due to photosyn-

thesis on the sediment surface. Furthermore, in most coastal sediments studied so far, the oxic and anoxic regions are separated by a "sub-oxic zone" which has neither sulfide nor oxygen present (Jørgensen, 1988). In this zone there is increasing evidence sulfide is oxidized without molecular oxygen but with alternate electron carriers such as iron, nitrate, nitrite, and oxidized manganese. At hydrothermal vents emitted sulfide is rapidly diluted, and chemically oxidized, upon contact with oxygenated ambient seawater. Thus bacteria dependent on the oxidation of reduced inorganic sulfur compounds for energy production must cope with intermittent supplies of sulfide and oxygen in these marine environments.

Free-living sulfur-oxidizing chemoautotrophs have evolved a number of adaptations in response to this fluctuating supply (Jørgensen, 1982). These include motility and chemotaxis, with the ability to track the oxygen-sulfide zone, e.g., Beggiatoa sp., or attachment to a surface in the flow of both sulfide and oxygen, e.g., Thiothrix sp. Some species pack a "lunch box" of elemental sulfur, an intermediate in the oxidation of sulfide, to be used as an energy source or as an electron acceptor when sulfide or oxygen are depleted, respectively (Nelson and Castenholz, 1981). A novel strategy, employed by Thiovulum sp., is to create its own interface by forming veils over decaying organic matter, trapping sulfide from below and oxygen from above.

Associating with a eucaryote may be viewed as another adaptation by aerobic sulfur bacteria to "bridge" oxic-anoxic interfaces. This suggests why symbiotic associations involving so many different hosts and bacteria have evolved. Sulfur-oxidizing chemoautotrophs can assure the simultaneous supply of necessary substrates by jumping on any organism that is either big or active enough to span the discontinuity of sulfide or oxygen (e.g., in sediments) or that can pump water from both oxic and anoxic sources (e.g., in turbulent flow environments). Thus for the examples discussed above, living with a partner that can (1) move faster and therefore more efficiently track a fluctuating oxic/anoxic interface (nematodes, oligochaetes), (2) sit in the

flow of anoxic and oxic water (vestimentiferans), (3) span with their body length the oxic/anoxic boundary (pogonophorans), or (4) create their own interface by burrowing in sediments and/or pumping water from both anoxic and oxic environments (bivalves), make sense in light of the need for substrates typically available from anoxic and oxic environments. While reciprocal benefits must accrue for the establishment of stable intracellular inhabitance, the use of a eucaryote for the simultaneous provision of substrates like sulfide and oxygen seems the likely scenario for the initiation of these associations. By associating with eucaryotes, the range of habitats is thus expanded for sulfur-oxidizing chemoautotrophs.

Viewed in this way, it is expected that sulfur-oxidizing chemoautotrophs will have evolved symbiotic associations widely in nature and might be expected in or on virtually any type of eucaryote living in habitats in which sulfide and oxygen are both available. As detailed above, symbioses with sulfur-oxidizing chemoautotrophs have already been demonstrated for species of five phyla. Circumstantial evidence presented indicating that similar symbioses in varying stages of evolution occur for many more species, including a protist, further suggest that this is the case. In many of these associations, the bacteria do not appear to provide any source of nutrition to the host, but rather use their host as a well-positioned or moving surface.

Furthermore, the recent discovery of symbioses between marine invertebrates and methanotrophs from deep-sea sediments and hydrothermal vents further supports the idea that these symbioses initiate as an adaptation by bacteria to obtain substrates from both oxic and anoxic zones (see review: Cavanaugh, 1992). Methanotrophs are bacteria which use methane as their main source of energy and carbon, and oxygen as the terminal electron acceptor. Like sulfide, methane is typically produced in reducing environments, e.g., in reducing sediments by methanogens, obligately anaerobic bacteria, or by abiogenic processes in hydrothermal vents (Schoell, 1988). Thus methanotrophs must also cope with the potentially fluctuating supply of substrates from mutually exclusive environments, and therefore can benefit from associating with eucaryotes which can "bridge" oxic/anoxic interfaces.

Overall the benefits afforded both host and symbiont suggest that symbioses with chemosynthetic bacteria will have evolved widely in nature. The relatively recent discovery of invertebrate-chemoautotroph associations suggests it would be productive to widen the search for these types of symbioses as well as other symbioses between eucaryotes and procaryotes with diverse metabolic abilities. The discovery of these partnerships will expand our understanding of both the array and complexity of biodiversity.

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