

Thiourine is a biomarker of sulfide-based symbiosis in deep-sea bivalves

Abstract—A simple biochemical approach for demonstrating the presence of symbionts in deep-sea bivalves and for discriminating between thiotrophic and methylo-trophic symbioses is described. Correspondence analysis (CA) of the free amino compound composition of nine bivalve species living in hydrothermal vents and cold seeps successfully discriminates symbiotic species from nonsymbiotic ones, and sulfur-oxidizing symbionts from methylo-trophic symbionts. CA was also used to infer the metabolism of *Bathymodiolus azoricus*, *B. boomerang*, and two new species of Mytilid and Vesicom-yid from the Barbados. These results were consistent with the evidence obtained by ultrastructural observations of the gills and enzymatic studies, and show that CA of amino acid profiles might be a useful tool to determine the type of endosymbionts present in deep-sea bivalves. Among all the free amino acids, thiourine appears as the main discriminating one and is proposed as a biomarker of sulfide-based endosymbiosis in deep-sea bivalves from hydrothermal vents and cold seeps.

Intensive exploration of the deep-sea floor has shown that symbiosis between chemolithoautotrophic or methylo-trophic bacteria and marine invertebrates is a general feature of sulfide-rich habitats, including hydrothermal vents and cold seeps, as well as in hydrocarbon seepages, anoxic basins, etc. (for review see Cavanaugh et al. 1987; Fiala Médioni and Felbeck 1990; Fisher 1990; Childress and Fisher 1992). Two main sources of energy are known to support life in such environments: sulfide and methane, the latter chemical often being a source of carbon as well. The bacterial symbionts use the energy derived from the oxidation of these reduced compounds to fix carbon dioxide and to provide the host with organic matter. Bivalves, often the dominant organisms in these communities, contain abundant intracellular symbionts in their gills. The members of the Vesicom-yid family are in strict association with sulfur-oxidizing bacteria, whereas Mytilids can house sulfur-oxidizing or methylo-trophic bacteria (for review see Fisher 1990; Childress and Fisher 1992; Nelson and Fisher 1995). The more general term methylo-trophic will be used instead of methanotrophic in this note. Recently, mussels containing both types of bacteria were described in cold seeps in the Gulf of Mexico (Fisher et al. 1993) and in hydrothermal vents from the mid-Atlantic ridge (Distel et al. 1995).

Several approaches, including microscopy, enzyme biochemistry, and stable isotope ratios, are currently used to demonstrate the presence of the endosymbiotic bacteria and to identify their source of energy. However, each type of approach has its own limitations and none of these approaches are sufficient when used alone (for review see Fisher 1990). Moreover, different techniques are required to determine whether endosymbionts are chemoautotrophic sulfur oxidizers or methylo-trophs.

A preliminary study of three deep-sea symbiotic Mytilids

has suggested that the free amino acid composition of their gills presents some particularities that might be related to the metabolic activity of their symbionts (Pruski et al. 1997). In order to verify this observation, we compared the free amino acid composition of nine deep-sea symbiotic bivalves and determined the source of energy used by the symbionts of four of these species.

Sample collection—Nine deep-sea symbiotic bivalves were collected from hydrothermal vent and cold seep sites (data concerning sites and dates of collection are given in Table 1): the Mytilids *Bathymodiolus azoricus*, *B. boomerang*, *B. brevior*, *B. childressi*, *B. puteoserpentis*, *B. thermophilus*, and an undescribed species *Bathymodiolus* n. sp. (Cosel pers. comm.) from the Barbados, as well as two Vesicom-yid species *Calyptogena magnifica* and an undescribed species from the Barbados probably related to the genus *Vesicom-yia* (Métivier pers. comm.). Specimens of the nonsymbiotic mussel *Mytilus galloprovincialis* were collected in March 1994 in the littoral of the western Mediterranean (Banyuls sur mer, France).

Free amino acid extraction and quantification—Gill tissues were excised and immediately frozen and stored in liquid nitrogen until lyophilisation at the laboratory. An ethanolic extraction of the free amino acid pool was chosen because the pH is more suitable for the analysis of sulfur amino acids (unpubl. data). Free amino acid concentrations were measured by reverse phase high-performance liquid chromatography (HPLC) after derivatization with ortho phtaldialdehyde as previously described in Pruski et al. (1998). Compound identification and quantification were achieved by comparing their retention time with those of standards of known concentration.

Main features of the free amino acid composition of deep-sea symbiotic bivalves—Average concentrations of the free amino acid pool ranged from 346 $\mu\text{mol g}^{-1}$ dry weight (dw) for the nonsymbiotic mussel *Mytilus galloprovincialis* to 767 $\mu\text{mol g}^{-1}$ dw for *Vesicom-yia* sp. from Barbados (Table 2). The nonsulfur compound content of all the species studied was characterized by high glycine concentrations (from 18% to 50% of the total amino compounds), which were higher in symbiotic bivalves than in the nonsymbiotic *M. galloprovincialis*. Alanine, glutamic acid, and arginine were found at low concentrations, and the other amino acids were only present as traces (<1% of the total amino compounds). The sulfur amino compound content was of special interest: only three amino acids, taurine, hypotaurine, and thiourine were present in high amounts, and their proportions were significantly different among the species studied. Taurine was predominant in the nonsymbiotic mussel *Mytilus galloprovincialis* (64% of the total amino compounds), whereas the symbiotic species were separated in three groups ac-

Table 1. Sampling data for nine deep-sea symbiotic species from hydrothermal vents and cold seeps.

Species	Site	Location	Coordinates	Cruise and/or date of collection
<i>Bathymodiolus azoricus</i>	Menez Gwen	Azores	37°45'N, 31°32'W, 800 m	DIVA2 1994
	Lucky Strike	Azores	37°20'N, 32°17'W, 1700 m	DIVA2 1994
<i>Bathymodiolus boomerang</i>	Orenoque B	Southern Barbados prism	10°19'N, 58°37'W, 1950 m	DIAPISUB 1992–1993
<i>Bathymodiolus brevior</i>	White Lady	North Fiji basin	16°59'S, 173°54'E, 1960 m	STARMER 1991
<i>Bathymodiolus childressi</i>	Brine Pool	Gulf of Mexico	27°43'N, 94°30'W, 710 m	1995
	Bush Hill	Gulf of Mexico	27°46'N, 91°30'W, 560 m	1995
	Alaminos Canyon	Gulf of Mexico	26°21'N, 94°30'W, 2000 m	1991
<i>Bathymodiolus puteoserpentis</i>	Snake pit	Mid-Atlantic ridge	23°23'N, 44°56'W, 3500 m	MAR 1993
<i>Bathymodiolus thermophilus</i>	9°N	East Pacific rise	9°50'N, 104°17'W, 2530 m	HOT 1996
<i>Bathymodiolus</i> n. sp.	El Pilar	Southern Barbados prism	11°14'N, 59°20'W, 1100 m	DIAPISUB 1992–1993
<i>Calyptogena magnifica</i>	9°N	East Pacific rise	9°50'N, 104°17'W, 2530 m	HERO 1994 and HOT 1996
<i>Vesicomya</i> sp.	Orenoque B	Southern Barbados prism	10°19'N, 58°37'W, 1950 m	DIAPISUB 1992–1993

cording to their relative concentrations in thiotaurine. On the one hand, *Calyptogena magnifica*, *Vesicomya* sp., and *Bathymodiolus brevior* were characterized by high thiotaurine concentrations (average values represented from 28% to 45% of the total amino compounds) and low taurine and hypotaurine concentrations. On the other hand, *Bathymodiolus childressi* and *B. sp.* from Barbados were characterized by very low thiotaurine concentrations (1% of the total amino compounds) and moderate taurine and hypotaurine concentrations (20% and 10% of the total amino compounds, respectively). *Bathymodiolus thermophilus*, *B. boomerang*, and *B. azoricus* were in intermediate situation with moderate concentrations of taurine, hypotaurine, and thiotaurine, *B. thermophilus* being characterized by a high hypotaurine content compared to the two other Mytilids (Table 2). The proportions of the other sulfur amino compounds were low in all symbiotic species and did not differ significantly from those found in the nonsymbiotic mussel *M. galloprovincialis*.

Statistical analysis—For the data processing, we used correspondence analysis (CA). Although this method was originally devised for the analysis of contingency tables, we selected it for the analysis of concentration tables (bivalve species \times amino acid concentrations) owing to the properties of its underlying chi-square metrics. With CA, we obtained simultaneously optimal ordinations of the bivalves from the relative (not absolute) concentrations of the different amino acids in their tissues, and optimal ordinations of the amino acid from their relative concentrations in the different bivalves. A given amino acid and the bivalve, in which this compound was in particularly high concentration, were close to each other on a given axis when the associated eigenvalue is sufficiently high. Moreover, once the analysis has been performed, it is easy to position supplementary animals on the axes knowing their relative concentrations in amino acid.

We first analyzed with CA a table combining the individual concentration of 26 amino acids in 40 individuals belonging to six bivalves species: the thiotrophic species *Bathymodiolus thermophilus*, *B. brevior*, and *Calyptogena magnifica*, the methylo-trophic mussel *B. childressi*, the dual symbiont-bearing species *B. puteoserpentis* (see Table 3 for

references on symbiont metabolism), and the nonsymbiotic mussel *Mytilus galloprovincialis* (Fig. 1).

The separation of the individuals on the first factorial axis (48.8% of the total inertia) was mainly explained by the relative importance of taurine and thiotaurine, which were negatively correlated (52 and 34% of the variance, respectively). Therefore, this axis separated the shallow nonsymbiotic mussel *Mytilus galloprovincialis* from the symbiotic bivalves and, among the symbiotic species, the three bivalves with sulfur-oxidizing symbionts only (*Bathymodiolus brevior*, *B. thermophilus*, and *C. magnifica*) from *B. childressi*, which only housed methylo-trophic symbionts. The second factorial axis (28.5% of the total inertia) was explained by two groups of variables, thiotaurine-aurine and hypotaurine-glycine, which were negatively correlated. This axis separated the two sulfur-oxidizing symbiont-bearing Mytilids *Bathymodiolus brevior* and *B. thermophilus* from the other species. A trend for higher glycine content and lower taurine content with depth was observed, the abscissa of samples on the first factorial axis being correlated with the depth of sampling ($r^2 = 0.68$, $P = 0.05$). This trend seems to be confirmed by the fact that individuals of *B. childressi* from the shallow sites of Bush Hill and Brine Pool were clearly separated from those of the deepest site of Alaminos Canyon (Fig. 1).

Determination of the symbiont metabolism of *Bathymodiolus azoricus*, *B. boomerang*, and undescribed Mytilid and *Vesicomya* species from Barbados by correspondence analysis and conventional methods—The ordination of the amino compounds in CA shows that three amino compounds (taurine, hypotaurine, and thiotaurine) are of sufficiently restricted distribution to allow the discrimination of the deep-sea symbiotic bivalves from the shallow nonsymbiotic mussels and that thiotaurine is a specific component of sulfide-based endosymbiosis in deep-sea bivalves. Therefore, it is possible to use the distribution of these amino compounds in the gills to identify the energy source used by the symbionts of an unknown symbiotic bivalve. To demonstrate the accuracy of this hypothesis, we determined the symbiont metabolism of four species from their amino acid composition by positioning them as supplementary samples on the previous CA axes

Table 2. Concentrations of free amino acids and related compounds in the gills of 10 bivalve species. Nine of these species are symbiotic and live in hydrothermal vents and cold seeps, the other one is the symbiotic free mussel *Mytilus galloprovincialis* that lives in shallow habitats. At the right of the table (last five columns) are four species of previously unknown metabolism that were added as supplementary samples in correspondence analysis. Values are expressed in micromoles per gram of dry weight tissue (mean \pm standard deviation). Number of samples is indicated in brackets.

	Species, site					
	<i>M. galloprovincialis</i>	<i>B. brevior</i>	<i>B. childressi</i>	<i>B. childressi</i>	<i>B. puteoserpentis</i>	<i>B. thermophilus</i>
	Gulf of Lion (4)	N. Fiji Basin (4)	Shallow sites (8)	Alaminos canyon (3)	Snake pit (4)	EPR (7)
Sulfur amino acids						
Cysteine sulfinic acid	0.6 \pm 0.2	1.4 \pm 1.1	1 \pm 0.1	1.6 \pm 0.7	0.7 \pm 0.4	0.9 \pm 0.5
Cysteine	15.8 \pm 5.4	10.3 \pm 1.6	4.5 \pm 2.7	1.9 \pm 1.6	4.3 \pm 1.1	4.0 \pm 2.3
Hypotaurine	7 \pm 5.5	18.5 \pm 22.3	36.7 \pm 19.9	104.6 \pm 18.6	47.5 \pm 29.2	112 \pm 32.9
Methionine	0.2 \pm 0.1	0.7 \pm 0.3	8.6 \pm 3.6	0.1 \pm 0.1	1.1 \pm 0.5	2.9 \pm 0.9
Taurine	227.9 \pm 51.6	67.7 \pm 15.9	101.5 \pm 24	51.1 \pm 11.1	16.6 \pm 8.6	36 \pm 19.2
Thiotaurine	0.8 \pm 0.1	149.7 \pm 39	1.1 \pm 0.4	1.4 \pm 0.4	46.1 \pm 41	68.1 \pm 17.4
Other amino acids						
Aspartic acid	15.6 \pm 2.9	1.2 \pm 0.4	2.5 \pm 0.8	4.6 \pm 0.8	1.9 \pm 0.5	2.8 \pm 0.7
Glutamic acid	6.2 \pm 1.1	14.7 \pm 3.6	30.4 \pm 2.8	29.9 \pm 3.1	19.9 \pm 4.5	32.4 \pm 16.1
β -glutamic acid	0	0.1 \pm 0	0.3 \pm 0.2	0.3 \pm 0.1	1.1 \pm 0.3	0.8 \pm 0.3
Asparagine	0.3 \pm 0.6	0	0.7 \pm 0.2	0.4 \pm 0.1	0	2.6 \pm 1.1
Alanine	11.5 \pm 0.1	48.9 \pm 6	12.9 \pm 2.6	14.3 \pm 0.5	46.2 \pm 16.4	59.6 \pm 6.9
β -Alanine	0.5 \pm 0.5	0.9 \pm 0.9	0.5 \pm 0.1	0	4.2 \pm 2.8	4 \pm 1.4
Arginine	4 \pm 0.5	5.3 \pm 1	1.9 \pm 0.2	7.9 \pm 0.4	7.2 \pm 2.3	32.2 \pm 4.5
Glutamine	0.6 \pm 0.1	0.7 \pm 0.3	1.7 \pm 0.5	1.3 \pm 0.3	2 \pm 0.6	3.7 \pm 1.2
Glycine	50.2 \pm 20.2	113 \pm 31.3	169.2 \pm 31.5	132.7 \pm 28.9	117 \pm 29.4	307 \pm 34.4
Histidine	0.4 \pm 0.1	0.8 \pm 0.2	0.1 \pm 0	0.5 \pm 0	1.1 \pm 0.5	0.9 \pm 0.2
Isoleucine	0.2 \pm 0.3	1.1 \pm 0.4	0.2 \pm 0.3	0.4 \pm 0.1	1.9 \pm 1	2.8 \pm 0.9
Leucine	1.2 \pm 0.3	1.8 \pm 0.6	0.8 \pm 0.8	0.5 \pm 0.2	2.6 \pm 1.3	5.8 \pm 1.9
Lysine	0.5 \pm 0.1	1.4 \pm 0.5	0.6 \pm 0.8	0.9 \pm 0.1	2.5 \pm 1.3	3.7 \pm 1.3
Ornithine	0.4 \pm 0.2	0.4 \pm 0.1	0	0	0	0.3
Phenylalanine	0.6 \pm 0.1	0.9 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.2	1.4 \pm 0.5	3.0 \pm 0.9
Serine	1.4 \pm 0.2	2.6 \pm 1.1	2.4 \pm 0.7	1.6 \pm 0	5.3 \pm 1.8	8.2 \pm 1.7
Threonine	0.8 \pm 1.4	4.2 \pm 2.6	6.3 \pm 0.8	1.4 \pm 0.3	7.8 \pm 1.4	4.4 \pm 1.6
Tryptophane	0	0.2 \pm 0	4.6 \pm 1.7	0	0.8 \pm 0.4	0.5 \pm 0.2
Tyrosine	0.3 \pm 0.2	0.9 \pm 0.2	1.5 \pm 0.8	0.6 \pm 0.2	3.3 \pm 0.7	4.3 \pm 1.3
Valine	0.4 \pm 0.4	1.3 \pm 0.3	1.1 \pm 0.6	0.6 \pm 0	2.7 \pm 1.3	4.3 \pm 1.4
Total	345.9 \pm 74.2	445.4 \pm 55.5	394.6 \pm 55.7	364.3 \pm 40.7	347.2 \pm 102.7	700 \pm 77.7

and we also confirmed these results using conventional methods (i.e., TEM observations and diagnostic enzymes, Fig. 2 and Table 3).

Individuals of the Barbados Vesicomid were grouped on the CA axes with individuals of the thiotrophic clam *C. magnifica*, whereas individuals of the undescribed Barbados Mytilid were placed between the two groups constituted by the methylotrophic mussel *B. childressi* (Fig. 1). *B. azoricus* from the Menez Gwen site was placed close to the group of *B. childressi* from the shallow sites, whereas *B. azoricus* from the Lucky Strike site and *B. boomerang* paralleled the diagonal gradient of thiotaurine. The position of these four species on the plane defined by the two first factorial axes of CA suggests that *Vesicomya* sp. houses sulfur-oxidizing symbionts, whereas *Bathymodiolus* sp. from the Barbados contains methylotrophic symbionts. That *B. boomerang* displays a large distribution on the F1 \times F2 plane of CA indicates a high variability of the thiotrophic and methylotrophic activities, respectively, among individuals of this species. Samples of *B. azoricus* form two distinct groups according to their site of collection, one with a marked dom-

inance of the methylotrophic metabolism in the Menez Gwen Mytilids, the other with a higher contribution of glycine in the deepest specimens from the Lucky Strike site (the same discrimination with depth has been observed for *B. childressi*).

The gill cells of the Vesicomid from the Barbados housed small coccoid Gram negative bacteria that resembled the chemoautotrophic sulfur-oxidizing symbionts found in *Bathymodiolus thermophilus* (Fiala Médioni et al. 1986), whereas larger symbionts that contained stacked intracytoplasmic membranes and resembled the methylotrophic symbionts found in the Mytilids from the mid-Atlantic ridge (Cavanaugh et al. 1992) were present in the undescribed mussel from the Barbados. Both types of symbionts were found in the gill tissue of *Bathymodiolus azoricus* and *B. boomerang*. The presence of RuBP carboxylase, ATP sulfurylase, and APS reductase in *Vesicomya* sp. from the Barbados indicates that the small symbionts found in the gill tissues of this bivalve are chemoautotrophic sulfur oxidizer bacteria (Fig. 2, Table 3). Furthermore, all Vesicomids so far studied only house sulfur-oxidizing symbionts. The presence of two dis-

Table 2. Extended.

Species, site						
<i>C. magnifica</i> EPR (1994) (5)	<i>C. magnifica</i> EPR (1996) (5)	<i>B. azoricus</i> Menez Gwen (4)	<i>B. azoricus</i> Lucky strike (4)	<i>B. boomerang</i> Barbados (3)	<i>B. sp.</i> Barbados (4)	<i>V. sp.</i> Barbados (4)
0	0	1 ± 0.4	0.7 ± 0.3	2 ± 1.4	11.7 ± 10.4	0
0	0.9 ± 0.9	30.5 ± 10.6	12.9 ± 1.9	12.9 ± 9.8	4.9 ± 4.2	0
16.7 ± 9.8	25.0 ± 21.1	34.8 ± 9.1	28.8 ± 10.7	31.1 ± 20.5	52.8 ± 23.3	10.6 ± 8.8
3.7 ± 0.2	1.1 ± 1.2	0.8 ± 0.3	1.4 ± 0.7	0.9 ± 0.3	0.8 ± 0.8	1.2 ± 0
6.3 ± 2.1	38.6 ± 15.3	152.9 ± 36	96.0 ± 15	30.4 ± 15.9	104 ± 27.9	26.3 ± 11.6
157.8 ± 28.6	136.7 ± 29.8	16.3 ± 19.2	62.1 ± 54.6	69.6 ± 83	4 ± 1	345 ± 74.8
4.5 ± 0.3	1.4 ± 1.8	6.1 ± 0.8	4.3 ± 0.6	2.4 ± 1.8	4.6 ± 2.9	5.2 ± 0.4
15.5 ± 1.1	18.9 ± 6.9	29.7 ± 8.5	30.8 ± 8.5	22.9 ± 2.9	36.5 ± 14	28.1 ± 5.1
1.3 ± 0.1	0.3 ± 0.3	0.2 ± 0.2	0.4 ± 0.1	0.6 ± 0.4	0.6 ± 0.5	0.7 ± 0.6
0.7 ± 0.1	0	1.4 ± 1	0.7 ± 0.4	1.2 ± 0.3	0	0.7 ± 0.2
28.9 ± 4.3	81.6 ± 18.9	20.5 ± 3.3	32.3 ± 7.6	23.4 ± 3.9	24.8 ± 10	58.1 ± 9.4
0	0.1 ± 0.3	2.0 ± 0.6	7.4 ± 2.9	1.3 ± 0.4	0.7 ± 0.2	1.4 ± 0.7
5.6 ± 0.9	1.0 ± 2.1	11.1 ± 4.7	11.1 ± 1.4	8.9 ± 6.2	14.3 ± 7.2	5.0 ± 3.4
0.5 ± 0	0	4.6 ± 1.3	2.4 ± 1	1.5 ± 0.3	2.4 ± 1	1.2 ± 0.2
60.1 ± 7.5	154.2 ± 43.8	198 ± 46.9	241.2 ± 31.7	198.4 ± 43	249.7 ± 120.4	253.2 ± 21.9
5.3 ± 0.5	1.2 ± 1.4	0.8 ± 0.1	1.1 ± 0.4	1.4 ± 0.7	1.5 ± 1.2	2.8 ± 0.3
4.3 ± 0.3	0.5 ± 0.5	0.9 ± 0.3	1.7 ± 0.6	1.4 ± 0.4	1.5 ± 1.5	5.2 ± 1.8
5.5 ± 0.3	2.1 ± 2.2	2.1 ± 0.5	3.3 ± 1.5	2.6 ± 0.7	1 ± 0.3	2.7 ± 0.5
6.5 ± 0.5	0.9 ± 0.8	1.5 ± 0.6	2.4 ± 0.8	2.2 ± 0.6	2.6 ± 2.7	2.3 ± 0.2
0	0	0	0	0.4 ± 0.5	0	0
3.3 ± 0.2	0.2 ± 0.3	0.7 ± 0.3	1.6 ± 0.6	1.1 ± 0.3	1.2 ± 1.4	0.8 ± 0.2
6.4 ± 1	8.5 ± 1.8	2.7 ± 0.3	3.6 ± 1	3.2 ± 1.1	3.1 ± 2.9	6.2 ± 1.6
5.2 ± 0.5	0.5 ± 1.2	2.5 ± 0.4	3.5 ± 0.8	3 ± 1.2	2.8 ± 2.9	2.2 ± 0.5
0	0	0.2	0.3 ± 0.1	0.2 ± 0.3	0.3 ± 0.3	0
4.3 ± 0.2	0.8 ± 0.8	1 ± 0.2	1.7 ± 0.7	0.9 ± 1.1	1.7 ± 1.8	2.7 ± 0.6
4.6 ± 0.4	1.5 ± 1.8	1.4 ± 0.3	2.5 ± 0.8	1.7 ± 0.4	2 ± 2.3	4.9 ± 1.6
346.6 ± 28.2	476.2 ± 73.7	526 ± 76.5	549.8 ± 98.9	464 ± 73.3	552.4 ± 233.3	766.5 ± 111.7

Table 3. Evidence of symbiont activity in nine bivalve species from hydrothermal vents and cold seeps: enzyme activities and visualization of symbiotic bacteria by electron microscopy. Abbreviations: SO, sulfur oxidizing bacteria; ME, methylotrophic bacteria; MDH, methanol dehydrogenase; RuBP, ribulose biphosphate carboxylase; ATP, ATP sulfurylase; APS, adenosine 5' phosphosulfate reductase; TEM, transmission electron microscopy.

Species	Enzyme activities				TEM	References
	RuBP	ATP	APS	MDH		
Mytilidae						
<i>Bathymodiolus azoricus</i>	+	+	+	+	SO ME	#
<i>Bathymodiolus boomerang</i>	-	-	-	+	SO ME	#
<i>Bathymodiolus brevior</i>	+	+	+	-	SO	1, 2
<i>Bathymodiolus childressi</i>	-	-	-	+	ME	3
<i>Bathymodiolus puteoserpentis</i>	-	-	-	+	SO ME	4, 5
<i>Bathymodiolus thermophilus</i>	+	+	-	-	SO	6, 7, 8, 9, 10
<i>Bathymodiolus</i> n. sp.	-	-	-	+	ME	#
Vesicomysidae						
<i>Calyptogena magnifica</i>	+	+	-	-	SO	6, 8, 11
<i>Vesicomys</i> sp.	+	+	+	-	SO	#

A. F. this study, (1) Dubilier et al. 1998, (2) Pranal et al. 1995, (3) Fisher et al. 1993, (4) Cavanaugh et al. 1992, (5) Distel et al. 1995, (6) Felbeck et al. 1981, (7) Fisher et al. 1987, (8) Fisher et al. 1994, (9) Fisher et al. 1988, (10) Fiala-Médioni et al. 1986, (11) Fiala-Médioni and Métivier 1986.

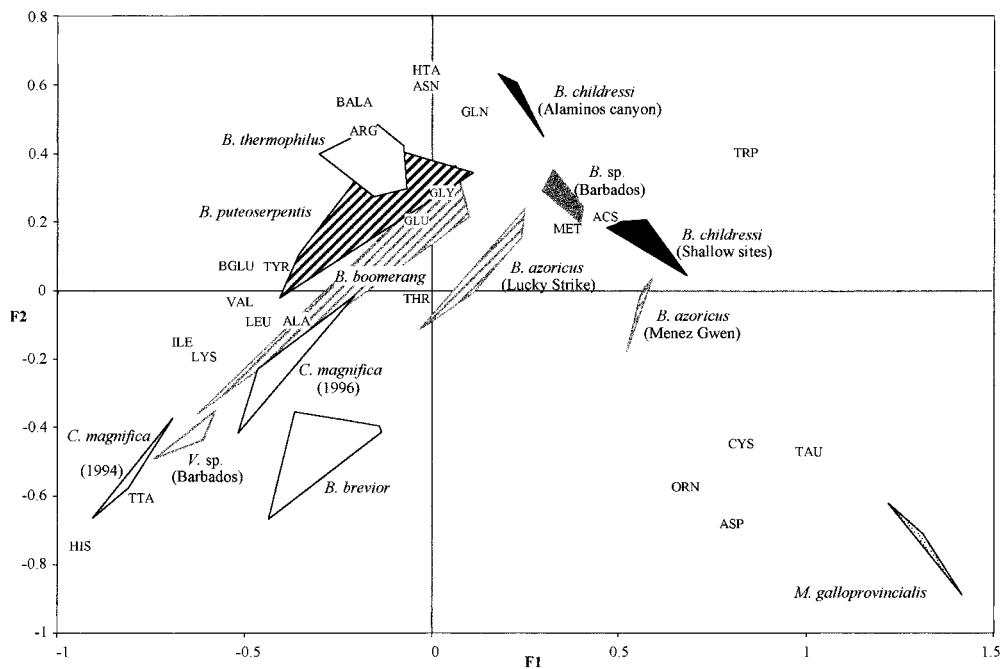


Fig. 1. CA plot for the data set showing relationships between species and individual amino acid concentration. Percentage of inertia accounted for: horizontal axis labeled F1 = 48.8%, vertical axis labeled F2 = 28.5%, total = 77.3% (arbitrary units). Polygons group together samples of a same species. In white, species with sulfur-oxidizing symbionts; in black, species with methylo-trophic ones; in hatching, dual symbioses; in dotted symbols, nonsymbiotic Mytilids. Projections of the four supplementary species are in gray. Abbreviations: ACS, cysteine sulfinic acid; ALA, alanine; ARG, arginine; ASN, asparagine; ASP, aspartic acid; BALA, β -alanine; BGLU, β -glutamic acid; CYS, cysteine; GLN, glutamine; GLU, glutamic acid; GLY, glycine; HIS, histidine; HTA, hypotaurine; ILE, isoleucine; LEU, leucine; LYS, lysine; MET, methionine; ORN, ornithine; PHE, phenylalanine; SER, serine; TAU, taurine; TTA, thiotaurine; THR, threonine; TRP, tryptophane; TYR, tyrosine; VAL, valine.

tinct types of symbionts in the gills of *Bathymodiolus azoricus*, together with the presence of the four enzymes tested, strongly suggests that, like the Mytilids from the mid-Atlantic ridge (Distel et al. 1995), *B. azoricus* also contains methylo-trophic and thioautotrophic symbionts (Fig. 2, Table 3). The cooccurrence of methanol dehydrogenase activity and of stacked internal membranes in the symbionts of the two Mytilids from the Barbados indicates methylo-trophic activities. However, in *B. boomerang*, the two distinct types of symbionts are present, contrary to *Bathymodiolus* sp. where only symbionts with intracytoplasmic bacteria are observed. It may be noticed that the absence of RuBP carboxylase, ATP sulfurylase, and APS reductase activities in *B. boomerang* is not incompatible with the cooccurrence of methylo-trophic and thio-trophic symbionts, but may be explained by an inactivation of the sulfur-oxidizing activity. Such an inactivation was observed in mussels from the mid-Atlantic ridge, where no enzymes diagnostic of sulfide oxidizing metabolism were detected (Cavanaugh et al. 1992), and where the coexistence of methylo-trophic and thioautotrophic bacteria was demonstrated either by TEM visualization or by in situ hybridization based on 16S rRNA sequencing (Distel et al. 1995).

In view of these results, it appears that the position of these four symbiotic species as supplementary samples on

the F1 \times F2 plane of our CA is consistent with their symbiont metabolism, confirming that correspondence analysis of amino acid concentration tables might be a useful tool to determine the type of endosymbionts present in deep-sea bivalves.

Discussion—Correspondence analysis of the species \times amino acids matrix groups the symbiotic bivalves according to the type of metabolism of their symbionts, independently of their taxonomical relationships and to the geographical location of the sites (Fig. 1). The analysis (1) successfully separates the symbiotic species from the non-symbiotic ones and (2) makes a clear distinction between the sulfur-oxidizing symbiotic activity and the methylo-trophic activity (Fig. 1). Correspondence analysis also allowed the determination of the symbiont metabolism of *Bathymodiolus azoricus*, *B. boomerang*, and the undescribed Mytilid and Vesicomid from the Barbados. This discrimination is explained by species-specific differences in relative abundance of the main sulfur amino acids (taurine, thiotaurine, and hypotaurine) according to the metabolism of their endosymbiotic bacteria or to the absence of endosymbionts. The nonsymbiotic *Mytilus galloprovincialis* is characterized by a high taurine content. In the symbiotic bivalves, the preponderance of glycine over taurine increas-

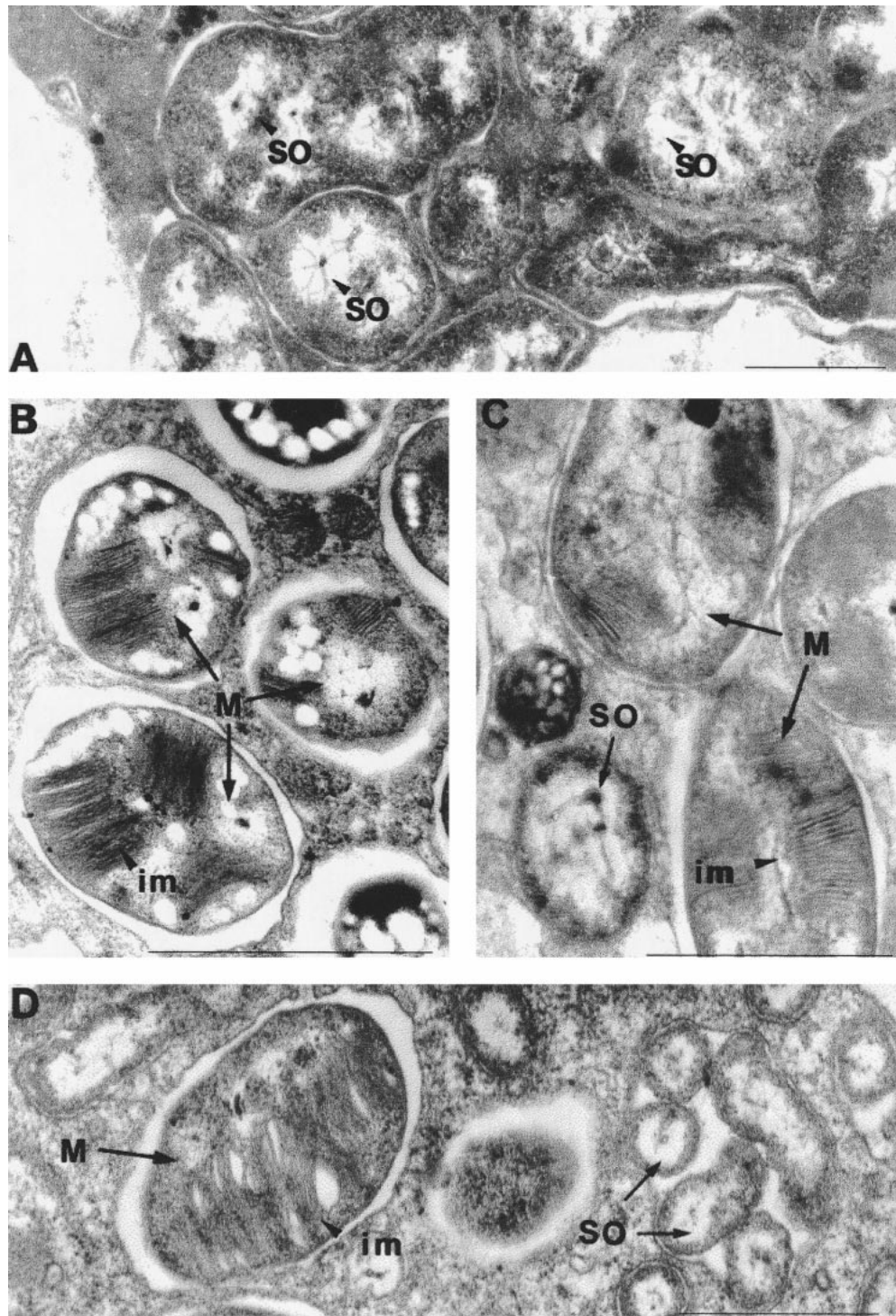


Fig. 2. Electron microphotographs of (A) *Vesicomya* sp. from the Barbados, (B) *Bathymodiolus* sp. from the Barbados, (C) *Bathymodiolus boomerang* from the Barbados, and (D) *Bathymodiolus azoricus* from the Azores. Scale bar = 1 μ m. Abbreviations: SO, sulfur-oxidizing-like symbiont; M, methylo-trophic-like symbiont; im, stacked internal membranes.

es with depth of collection (Table 2). Taurine and glycine are known to be the major organic effectors in osmoregulation in many marine invertebrates (Bayne et al. 1976; Bishop et al. 1983). The high glycine content of the deepest

symbiotic bivalves might be explained by depth-related differences in the relative bioavailability of taurine and glycine as discussed in Pruski et al. (2000).

Among these amino acids, thiotaurine appears as the

main discriminating amino acid of the symbiotic species, as shown by its high contribution to the two first factorial axes of CA. Large amounts of thiotaurine were found only in sulfur-oxidizing bearing bivalves. This is in agreement with results obtained on other thiotrophic species: the vestimentiferan *Riftia pachyptila* (Albéric 1986), the vesicomyid *Calyptogena phaseoliformis* (Albéric and Boulègue 1990), the mytilid *Bathymodiolus elongatus* (Pranal et al. 1995), the gastropods, *Ifremeria nautilei*, and *Alvinoconcha hessleri* (Pranal 1995). Therefore, thiotaurine can be proposed as a general biochemical marker of sulfide-based endosymbiosis between bacteria and marine invertebrates.

Although the presence of thiotaurine in *Bathymodiolus thermophilus* is in accordance with the thiotrophic activity of its symbionts, it might be suggested that the contribution of the sulfur-oxidizing symbionts to the nutrition of their host is lower in this species than in the two other thiotrophic bivalves studied (*B. brevior* and *C. magnifica*). In *B. thermophilus*, thiotaurine contribution to the free amino acid pool is indeed relatively low and the hypotaurine content particularly high. Several authors have previously hypothesized that *B. thermophilus* was less dependent than other species on its sulfur-oxidizing symbionts, since it contained no elemental sulfur (Fisher et al. 1987), and since RuBP carboxylase activities were low (Felbeck et al. 1981; Fisher et al. 1988).

The origin of the thiotaurine found in thiotrophic species is unknown. The fact that there is less hypotaurine (the sulfenic analogue of thiotaurine) in the gills of the sulfur-oxidizing symbiont-bearing species than in the methylotrophic symbiont-bearing ones suggests that the metabolic route for thiotaurine synthesis could be through hypotaurine. This is in agreement with the processes of thiotaurine formation reported in the literature: (1) from hypotaurine, which is a by product of taurine metabolism (Cavallini et al. 1959); (2) from the enzymatic conversion of sulfinates (hypotaurine) to thiosulfinates (thiotaurine) (Sörbo 1957); and (3) from the transfer of sulfur between thiosulfenic and sulfenic compounds by nonenzymatic reversible transsulfuration reactions (De Marco and Luchi 1972).

Although the role of thiotaurine is not yet clearly determined, the large amounts of this unusual compound in the gills of *Calyptogena magnifica*, *Bathymodiolus brevior*, *B. thermophilus*, and *B. puteoserpentis* suggest that this amino acid may have an important function in sulfide metabolism. Thiotaurine might bind sulfide in a nontoxic form to protect the host's tissues from its toxicity. Furthermore, since thiotaurine can be involved in reversible transsulfuration reactions, it might participate to the transport of reduced sulfur from the environment to the symbionts (Albéric 1986; Albéric and Boulègue 1990) or to its temporary storage as suggested for elemental sulfur in several clams (Vetter 1985). The abundance of thiotaurine is restricted to the gill tissues where the sulfur-oxidizing symbionts are located. Indeed, the thiotaurine content in the mantle tissues is very low in all the symbiotic bivalves (from 0.59% to 1.85% of the total free amino compounds; Pruski unpubl. data). This observation, together with the dominance of hypotaurine (the sulfenic form) over thiotaurine in the bivalves with methylotrophic bacteria only, suggests that thiotaurine may be in-

involved in energy metabolism (transport or/and storage of reduced sulfur) rather than in detoxification of hydrogen sulfide.

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