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C_1 bacteria in the water column of Chesapeake Bay, USA. II. The dominant O_2- and H_2S-tolerant methylotrophic methanogens, coenriched with their oxidative and sulphate reducing bacterial consorts, are all new immunotypes and probably include new taxa

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ABSTRACT: Methylotrophic methanogens were readily enriched with monomethylamine (MMA) in water samples obtained from throughout the stratified but oxygenated water column of Chesapeake Bay, USA. Of the 3 different populations of methylotrophic methanogens enriched, O_2- and H_2S-tolerant methanogens enriched in bacterially reduced seawater in glass were twice as prevalent as those enrichments either reduced chemically in glass or reduced bacterially in semipermeable polycarbonate flasks. Thirty-three obligately anaerobic, but O_2- and H_2S-tolerant, methanogenic cultures from the dominant group were fingerprinted immunologically using antibody probes. The antigenic fingerprints divided these cultures into 4 clusters. One cluster was related to *Methanosarcina barkeri* R1M3, a second was related to *M. mazei* S6, and a third was related to other species of *Methanosarcina*. None of these were identical to known species and can be considered new immunotypes. These new immunotypes, related to described species, were found throughout the water column. A fourth cluster was unrelated to any extant genus of methanogens, was absent from surface waters, and was restricted to the pycnocline and deeper waters. This group of new methanogens constituted 24 % of the pycnocline cultures and 39 % of those from bottom waters. Stratification, which is a prerequisite for the development or concentration of methane-cycle bacteria in the pycnocline, apparently allows unique water-column methanogens to selectively enrich in particulates in the pycnocline that can increase in bottom waters before sedimentation. The observed distribution of known and new taxa of methanogens in the 3 water layers of Chesapeake Bay is illustrated by a model.

INTRODUCTION

Methane accumulates in the mixing layer of the upper ocean and forms a peak in the seasonal pycnocline that is well documented (Swinnerton et al. 1969, Brooks & Sackett 1973, Seiler & Schmidt 1974, Scranton & Brewer 1977, Burke et al. 1983, among others). The seasonal pycnocline cuts off the upper productive mixing layer of the sea from deeper water and benthic processes in the sediments, and greater than 90 % of nutrient regeneration occurs in or above the pycnocline (Baker et al. 1985, Gardiner et al. 1985). In this stratified condition, microdebris with its associated aerobic and anaerobic bacteria could accumulate in the pycnocline, making benthic processes possible in the upper water column. Benthic processes tightly

coupled to photosynthesis and grazing in the pycnocline could be the site of upper ocean methane production and consumption (Sieburth 1983, 1987, 1988, 1991).

The first bacterium participating in the postulated upper water-column methane cycle to be cultured, isolated and characterized, was the methane-oxidizer *Methylomonas pelagica* (Sieburth et al. 1987). This bacterium was readily enriched from well-stratified gyre waters of the Sargasso Sea, but not from well-mixed slope waters. Later attempts to enrich C_1 bacteria with other C_1 substrates included monomethylamine MMA), whose enrichments quickly became suboxic, anoxic, and often contained hydrogen sulphide and methane (Sieburth 1987, 1988, 1989, 1993). We had serendipitously stumbled upon a procedure for enriching obligately anaerobic, oxygen-tolerant methanogens from oxygenated seawaters with their necessary aerobic and anaerobic bacterial consorts. This is apparently the same mechanism that algae which peak in the pycnocline use to enrich and maintain their methylotrophic C_1 bacterial consorts required for nutrient regeneration (Sieburth 1988). The source of MMA that enrich for C_1 bacteria in nature appears to be the osmoprotectant glycine betaine that degrades to release the methylated amines trimethylamine, dimethylamine and monomethylamine (Sieburth & Keller 1988/89, Sieburth 1991).

In the first part of this study, we observed that methanogenic bacterial consortia (MBC) accumulated to form a peak in the pycnocline. We also used 3 different enrichment procedures to determine which was most effective in culturing methylotrophic methanogens. The addition of MMA to seawater samples in glass serum bottles enriched MMA-oxidizers that removed dissolved oxygen, while permitting poorly soluble oxygen and methane to persist in the headspace. This bacterial reduction in sealed glass bottles yielded double the number of positive enrichments obtained with either a chemically reduced medium in glass bottles used to obtain methanogens from anoxic sediments (Sowers & Ferry 1983), or by bacterial reduction in polycarbonate flasks which allowed methane, but not hydrogen sulphide, to accumulate. Methanogens enriched by chemical reduction up the estuary, were replaced seaward by the dominant O_2- and H_2S-tolerant methanogens that were enriched by bacterial reduction (Sieburth 1993). The objective of this part of the study was to use 33 cultures of the dominant O_2- and H_2S-tolerant methanogens to characterize water-column methanogens immunologically for the first time, and to determine the vertical distribution of these methanogen immunotypes in the 3-layered water column of Chesapeake Bay, USA.

Elucidation of complex microbial communities such as the components of our methanogenic enrichment cultures is difficult and time consuming when culture-isolation procedures are utilized. Furthermore, even when new isolates are successfully maintained in axenic cultures, their precise identification and classification require cumbersome techniques, including nucleic acid analyses (Balch et al. 1979, Boone & Whitman 1988). While these analyses are necessary for phylogenetic assignment, 'antigenic fingerprinting' using antibody probes provides useful data for the identification and classification of new isolates (Macario & Conway de Macario 1983) and for the characterization of strains in complex ecosystems (Macario & Conway de Macario 1988).

The extant species of methanogens were originally obtained from anoxic muds using chemically reduced media. All the water-column methanogens studied here were originally enriched by using MMA-oxidizing bacteria to reduce the originally oxygenated seawater sample, before subculture of the methanogenic cultures into a chemically reduced medium that would eliminate the aerobes that might interfere with the microscopic examination of the methanogens during antigenic fingerprinting. All the cultures so obtained appear to be distinct immunotypes. Of the methanogens enriched with their aerobic consorts from the pycnocline and bottom waters, 24 and 39 %, respectively, are unrelated to known taxa and must be new species or genera. A model for the distribution of the known and new taxa of methanogens in a 3-layered system, based on our results for Chesapeake Bay and its adjacent shelf water, is proposed.

MATERIALS AND METHODS

Sampling. Chesapeake Bay was chosen as the study site for sampling in early May during 1986 and 1987. Station locations are shown in Fig. 1 of Sieburth (1993). Stns 904 and 858, above and below the Chesapeake Bay Bridge, respectively, were occupied between 4 and 9 May 1986 on cruise PROPHOT XVIII by the RV 'Cape Henlopen' (University of Delaware), the others were occupied between 9 and 12 May 1987 on cruise CORSAIR XII by the RV 'Cape Hatteras' (University of North Carolina). Continuously recording conductivity, temperature, depth profilers (CTDs) were used to obtain vertical profiles in the water column for density (σ_t), salinity (‰); temperature (°C), and oxygen (mg l^{-1}). The density profiles used to pick the depths for the 3 water layers at each station are shown in Fig. 6 of Sieburth (1993). Water samples were then collected in 5 l Niskin bottles attached to the CTD rosette at selected depths above (upper, mixed layer), within, and below the pycnocline (bottom water layer) for the enrichment of bacterially reduced methylotrophic methanogens that were both O_2- and H_2S-tolerant.

Stable enrichment cultures of methanogens. During the incubation of the bacterially reduced enrichments for the most probable numbers (MPN) of methanogenic bacteria (Sieburth 1993), bottles that were positive for the production of methane were then used as a source of inocula for a chemically reduced medium in 50 ml serum bottles (Sowers & Ferry 1983) in order to enhance the growth of the anaerobic methylotrophic methanogens over their aerobic bacterial consorts. Occasionally, the antibiotics vancomycin and penicillin were also used at a concentration of 25 µg each per ml of medium (Sowers & Ferry 1983) in order to select for the growth of methanogens. The methanogenic MPN enrichment cultures that grew upon transfer to the same medium and produced large quantities of methane were selected for preparation for antigenic analysis (Macario & Conway de Macario 1983). Each culture was fixed for 0.5 h with the addition of formaldehyde (1% final concentration) and then centrifuged for 10 min at 10 000 rpm. The pellets were resuspended in 5.0 ml of 1% formaldehyde in 0.85% NaCl and stored at 4°C until they were analyzed. Representative methanogenic enrichment cultures were studied immunologically using a comprehensive panel of calibrated antibody probes (Macario & Conway de Macario 1983, 1985) for the reference methanogens (Balch et al. 1979, Boone & Whitman 1988) listed in Table 1.

Antigenic fingerprinting. Indirect immunofluorescence and quantitative slide immunoenzymatic assay (SIA) were applied as described previously (Conway de Macario et al. 1986b). The antigenic fingerprint of each isolate was determined, and quantitative comparisons of the isolates' fingerprints with each other and with reference fingerprints were carried out as described previously (Macario & Conway de Macario 1985). In addition to the standard 31 reference fingerprints (Macario & Conway de Macario 1983, 1988) others were used for comparative analysis. These additional antigenic fingerprints were those of halophiles (Conway de Macario et al. 1986a), *Methanococcoides methylutens* (Sowers & Ferry 1983), *Methanosarcina acetivorans* (Sowers et al. 1984), strain SF1 (Mathrani & Boone 1985), *Methanohalophilus mahii* (Paterek & Smith 1985), *Methanogenium aggregans* (Ollivier et al. 1985), *Methanogenium thermophilicum* (Zabel et al. 1985), *Methanogenium bourgense* (Ollivier et al. 1986) and other bacteria (Touzel et al. 1985, Macario et al. 1987, Paterek & Smith 1988) including marine mesophilic and thermophilic methanogens.

The following indexes were calculated for each pair of antigenic fingerprints we compared: cumulative antigenic fingerprint (CAF); CAF percent; cumulative difference (CD); and reactivity percent (*r* percent) (Macario & Conway de Macario unpubl.).

CAF was calculated using the equation:

$$\mathrm{CAF} = \left(n \times \sum_{n-1}^{n}\right) - \left(n' \times \sum_{n'-1}^{n'}\right)$$

where n = number of matching positions at which the 2 fingerprints under comparison, usually the isolate's and reference fingerprints, show a value greater than zero; and $\sum_{n-1}^{n}$ = the sum of the values at these positions in the isolate's fingerprint. The expressions n' and $\sum_{n'-1}^{n'}$ have the same meaning as

Table 1. Reference methanogens used to prepare calibrated antibody probes for antigenic fingerprinting[a]

Reference methanogen	Species	Strain
1	*Methanobrevibacter smithii*	PS
2	*Methanobacterium formicicum*	MF
3	*Methanosarcina barkeri*	MS
4	*Methanobacterium bryantii*	MoH
5	*Methanobacterium bryantii*	MoHG
6	*Methanosarcina barkeri*	R1M3
7	*Methanospirillum hungatei*	JF1
8	*Methanobrevibacter ruminantium*	M1
9	*Methanobrevibacter arboriphilus*	DH1
10	*Methanobrevibacter smithii*	ALI
11	*Methanobacterium thermoautotrophicum*	GC1
12	*Methanobacterium thermoautotrophicum*	ΔH
13	*Methanococcus vannielii*	SB
14	*Methanococcus voltae*	PS
15	*Methanogenium marisnigri*	JR1
16	*Methanosarcina barkeri*	227
17	*Methanogenium cariaci*	JR1
18	*Methanosarcina mazei*	S6
19	*Methanosarcina barkeri*	W
20	*Methanosarcina thermophila*	TM1
21	*Methanobrevibacter arboriphilus*	AZ
22	*Methanobrevibacter arboriphilus*	DC
23	*Methanomicrobium mobile*	BP
24	*Methanothermus fervidus*	V24S
25	*Methanolobus tindarius*	Tindari
26	*Methanococcus maripaludis*	JJ
27	*Methanosphaera stadtmanae*	MCB3
28	*Methanoplanus limicola*	M3
29	*Methanococcus thermolithotrophicus*	SN1
30	*Methanothrix soehngenii*	Opfikon
31	*Methanothrix* sp.	CALS-1

[a] Descriptions of methanogens are given in Balch et al. (1979) and Boone & Whitman (1988), while those of antibody probes and the antigenic fingerprinting method are presented in Macario & Conway de Macario (1983, 1988)

above, but refer to positions in the isolate's fingerprint showing a value greater than zero that correspond to a zero value position in the reference fingerprint. CAF percent is the percentage of the reference CAF represented by the isolate's CAF. CD was calculated as follows:

$$CD = \frac{\sum_{nd-1}^{nd}}{nd}$$

where $\sum_{nd-1}^{nd}$ = the sum of the differences between the values in those positions at which the 2 fingerprints under comparison show values greater than zero, ignoring the sign; and nd = number of positions showing values greater than zero in both fingerprints. Reactivity percent, r, is the percent of the reaction of the isolate with the probe for a reference methanogen, considering the reaction of the latter with its probe is equal to 100.

The methanogenic enrichment cultures prepared for antigenic analysis as described above were examined using oil immersion with phase contrast light microscopy at 1000× with a Zeiss photomicroscope. The same cultures were also tested with the Gram stain and examined under oil immersion (1000×) with brightfield microscopy for their Gram reaction. Representative cultures were examined in thin sections by transmission electron microscopy as described previously (Johnson & Sieburth 1982).

RESULTS

Distribution of O_2- and H_2S-tolerant methanogens

The station locations where the methanogen cultures were obtained are shown in Fig. 1 of Sieburth (1993). The density plots (σ_t) for these stations are shown in Fig. 6 of Sieburth (1993), and were used to detect the pycnocline and to select the depths for sampling the upper layer, pycnocline and bottom layer of water. The distribution of the 3 different methanogen populations cultured, including the dominant O_2- and H_2S-tolerant methanogens obtained with bacterial reduction that are studied here, are shown in Fig. 7 of Sieburth (1993).

Antigenic fingerprinting of methanogens

Eleven methanogenic enrichment cultures that underwent successive transfers in chemically reduced medium from the 1986 study and 22 cultures from the 1987 study were prepared for immunologic analysis. The source of these cultures and their antigenic relatedness to reference methanogens are summarized in Table 2. All the methanogenic enrichment cultures were Gram-negative, being coccoid cells 1 to 3 µm in diameter and occurring as single, paired, or packets of cells. Electron photomicrographs of thin sections

Table 2. Source of the methanogenic enrichment cultures used in the antigenic fingerprinting according to station and water layer for 1986 and 1987

Culture no.	Stn[a]	Water layer[b]	Antigenically related to reference methanogen number[c]
		1986	
60–34	858	Lower	6
60–69	858	Lower	18
60–72	858	Lower	0[d]
60–116	904	Pycnocline	0
60–119	16 (sediment trap)	Lower	0
60–120	16 (sediment trap)	Upper	20
60–121	16 (sediment trap)	Upper	18
60–122	16 (sediment trap)	Lower	0
125–14	904	Pycnocline	18
160–16	858	Pycnocline	18
160–26	858	Pycnocline	6
		1987	
198	9	Lower	0
227	13	Upper	3
241	16	Upper	18
243	16	Upper	6
252	16	Pycnocline	6
254	16	Pycnocline	0
257	16	Lower	18
385	16	Upper	6
386	16	Lower	6
388	1	Upper	18
389	2	Upper	18
391	2	Upper	6
392	2	Lower	6
393	15	Upper	6
396	16	Lower	20
398	16	Lower	0
399	1	Upper	18
403	11	Lower	6
410	2	Upper	18
421	5	Lower	19
506	16	Pycnocline	6
507	16	Pycnocline	18

[a] See Fig. 1 in Sieburth (1993)
[b] Upper layer = 0 to 4 m; pycnocline = 6 to 12 m; and lower layer = 14 to 20 m
[c] See 'Materials and methods' for a complete list of numbered reference methanogens
[d] 0 = no antigenic relatedness to any reference methanogen

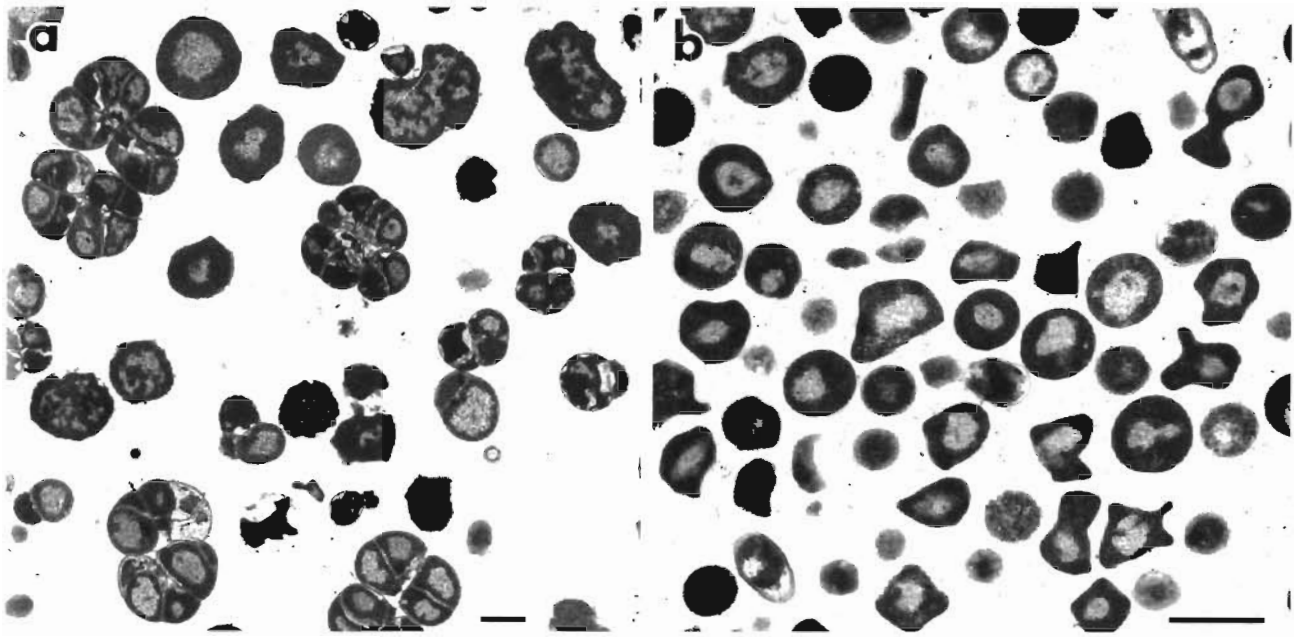

Fig. 1. The characteristic ultrastructure of methanogens as observed in thin sections of cells examined with transmission electron microscopy. (a) A culture closely related to *Methanosarcina mazei* antigenically (Culture no. 60–121), showing typical sarcina-like packets of cells and other morphological forms known to be part of the life cycle of this species (Robinson 1986). (b) A culture completely unrelated to other methanogenic species (Culture no. 198) showing coccoidal and irregularly shaped cells. Scale bars = 1.0 µm

for 2 strains, one antigenically related to the reference species *Methanosarcina mazei* S6, and one unrelated to known methanogens, are shown in Fig. 1a, b, respectively.

Quantitative comparisons of the fingerprints of the 33 methanogenic enrichment cultures with each other and with a wide variety of reference methanogens, showed the following: 7 cultures were antigenically unrelated to any of the reference organisms; and 26 cultures showed various degrees of relatedness with *Methanosarcina* spp. The antigenic fingerprints of the latter 26 isolates are summarized in Table 3, along with the most similar reference fingerprints. It is clear that Isolate 227 is antigenically related to *Methanosarcina barkeri* MS, Isolates 125-14, 257, 389 and 507 are related to *M. mazei* S6, Isolate 421 is related to *M. barkeri* W, and Isolates 60-120 and 396 are related to *M. thermophila* TM1. The other 18 isolates, however, show more complex antigenic relationships. A quantitative comparison of the antigenic fingerprints of these 18 isolates with the fingerprints of reference *Methanosarcina* spp. showed various degrees of relatedness to Reference methanogen 6, *M. barkeri* R1M3, and to Reference methanogen 18, *M. mazei* S6.

Assignment of a culture to an antigenic cluster defined around a reference *Methanosarcina* spp, was done when 2 or more of the 4 indexes (CAF, CAF percent, CD and *r* percent) indicated a stronger relationship with the reference sarcina defining the cluster than with the others. For example, Isolate 388 showed the largest CAF percent and *r* percent, and the smallest CD with respect to *Methanosarcina mazei* S6 and was, therefore, assigned to the antigenic cluster of this species. Isolate 60-34, on the other hand, showed the highest CAF percent and *r* percent and the lowest CD with respect to *M. barkeri* R1M3, therefore, it was assigned to this strain's cluster.

This immunologic analysis permitted us to divide the 33 methanogenic enrichment cultures into 4 clusters: those antigenically unrelated to reference methanogens; those related to *Methanosarcina barkeri* R1M3; those related to *Methanosarcina mazei* S6; and those related to a number of other *Methanosarcina* spp. The distribution of these 4 clusters in the 3 water layers of Chesapeake Bay is given in Table 4. The mean oxygen concentrations for these 3 layers show saturation in the upper mixing layer, some depletion in the apparent pycnocline, and a trend to suboxia in the bottom water layer, while the salinity increased with depth as expected. What is noteworthy, is that all 12 methanogenic cultures from the upper less-saline layer were related to known species of *Methanosarcina*, while 24 % of the cultures obtained from the pycnocline and 39 % of those from the bottom layer were unrelated to reference methanogens.

Table 3. Antigenic fingerprints of Chesepeake Bay methanogenic enrichment cultures displayed along with the most similar reference counterparts. The reference methanogens and their reactions with the antibody probes are in bold face, the culture enrichments and their reactions with the antibody probes are in plain face. See 'Materials and methods' for the list of probes and reference methanogens. Probes 1, 2, 4, 5, 7, 17 and 21 to 31 did not react with any cultures. Cultures 60-72, 60-116, 60-119, 60-122, 198, 254 and 398 did not react with any probes. Blanks = 0 (meaning no reaction)

Methanogen		Reaction with antibody probe no.					
Culture	Reference	3	6	16	18	19	20
	3	**4**					
227		2					
	6	**2**	**4**	**3**	**4**		**3**
391, 392, 393			2		1		2
385, 386			1		2		1
60-34			2		2		2
403		2			1		1
506		1			2		1
243					1		1
252		3			1		
160-26			2		1		
	18	**1**	**3**	**3**	**4**	**3**	**1**
60-69			1		1	1	1
160-16			2		2	2	1
60-121			2		2	2	2
241			1		3		2
388			1		2	2	1
399		1		2		2	
410		1				2	
125-14, 257					1		
389, 507					1		
	19					**4**	**1**
421						2	
	20	**1**	**3**	**3**	**2**	**4**	**4**
60-120							4
396							2

DISCUSSION

Immunologic analysis revealed 2 main groups of methanogens: one antigenically unrelated to any of the well-known reference species, and a second larger group related to *Methanosarcina* spp. That the majority of isolates were related to the methylotrophic genus *Methanosarcina* is likely due to the fact that they were enriched with MMA. Also, the great majority grew as single cocci. Although salty culture media and methylamines are known to promote single cocci growth (Boone & Mah 1987, Sowers & Gunsalus 1988), a minority of cultures grew as packets (Fig. 1a).

Cluster assignment was straightforward for a number of isolates, but for others detailed quantitative analysis was necessary. Seven isolates (241, 385, 386, 391, 392, 393 and 60-34) showed a low degree of antigenic similarity with *Methanococcoides methylutens*, in addition to their similarities to *Methanosarcina mazei* S6, *Methanosarcina barkeri* R1M3 and *Methanosarcina thermophila* TM1. However, none of these isolates was recognized by the probe for *Methanolobus tindarius* Tindari, which placed them apart from *Methanococcoides methylutens* (data not shown). Therefore none of the Chesapeake Bay isolates appear to be closely related to the single *Methanococcoides methylutens* strain obtained from a zooplankton tow in the Pacific that

Table 4. The apparent effect of the 3-layered structure of the water column of stratified Chesapeake Bay on the distribution of methanogenic immunotypes. *M.*: *Methanosarcina*

Water layer	$[O_2]$ (mg l^{-1})	Salinity (‰)	Methanogens (%)				
			New[a]	Antigenically related to:			
				M. barkeri R1M3	*M. mazei* S6	Other	Total[b]
Upper	8.3 (7.5–9.5)	10.3 (6.4–19.7)	0 (0/12)[c]	33 (4/12)[d]	50 (6/12)	17 (2/12)	100
Pycnocline	5.6 (3.5–7.2)	14.0 (9.4–23.9)	24 (2/8)	38 (3/8)	38 (3/8)	0 (0/8)	76
Lower	3.4 (0.8–6.2)	17.1 (9.7–27.1)	39 (5/13)	31 (4/13)	15 (2/13)	15 (2/13)	61

[a] Antigenically unrelated to reference methanogens
[b] Percent of cultures related to known, reference methanogens
[c] Number of cultures unrelated to reference methanogens/total cultures examined
[d] Number of cultures related to the reference methanogen shown above/total cultures examined

was enriched with a chemically reduced medium (Cynar & Yayanos 1991) which would have eliminated any aerobic bacterial consorts.

The immunologic data indicated that all cultures were immunotypes that differed from the reference methanogens. Cultures showing no antigenic relatedness with any of the reference methanogens are likely to be new species, perhaps examples of as yet undescribed genera or families. The origin of these methanogens, unrelated to known species, appears to be the pycnocline, and their incidence appears to increase in deeper water, due presumably to their selective enrichment on microparticulates that sink and degrade during sedimentation. These uncharacterized new methanogens may be the uncharacterized Archaea detected with molecular phylogenetic analyses by DeLong (1992). The methane-oxidizers from the stratified waters of Chesapeake Bay, that are dependent upon the methanogens for their substrate, also appeared to be new immunotypes (Sieburth et al. 1993).

We have previously reported that the dominant group of cultivable water-column methanogens are dependent upon their aerobic and anaerobic bacterial consorts to create reduced microsites in an otherwise oxygenated habitat (Sieburth 1993). Here we report that all the methanogenic cultures tested from this group are new immunotypes, and that the water in the pycnocline and bottom layer apparently contain new taxa. A model for the occurrence of new and known taxa of methanogens in the stratified and unstratified waters of the Chesapeake Bay system is suggested in Fig. 2. It is based on the data reported here as well as that of Sieburth (1993). Methanogenic species isolated and characterized from the marine habitat have all been obtained from anoxic sediment. Accordingly, the low populations of methanogens related to known taxa in the surface waters from the fresh waters of the Susquehanna River above Turkey Point, through the stratified upper bay waters to the shelf waters must have had their source in the riverene sediments resuspended by tidal and storm action. At Stn 1 in fresh water and at Stn 13 in full salinity shelf water there was no stratification and the waters were well mixed. At these stations there was a minimal methanogenic population that occurred only in the surface layer, where it was presumably carried seaward by the outgoing surface waters. The equally small population related to known species of methanogens in the stratified upper bay waters increased dramatically in the nutrient-rich pycnocline which also appeared to favor the growth of methanogens distinct from those in culture collections. An increase of these as yet uncharacterized methanogens from 24 % in the pycnocline to 39 % in bottom waters may show selective enrichment of the particle associated methanogens during sedimentation, since the MPN estimates showed no trend for an increase in methanogen populations in the bottom waters (Sieburth 1993). This working model for the distribution and nature of estuarine methanogens needs to be verified.

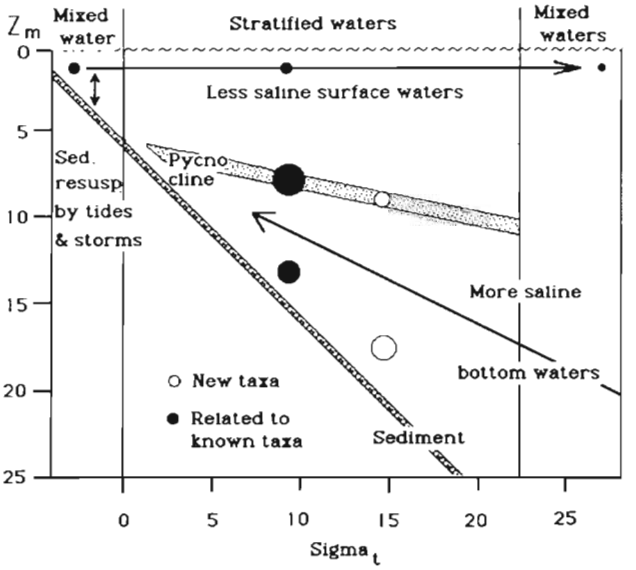

Fig. 2. A speculative model that attempts to show how the mixing and stratification zones in the Chesapeake Bay and shelf ecosystem could explain the distribution of methanogen taxa shown in Table 4. It is also based on the methanogen population data of Sieburth (1993). As methanogens which are identical to or related to known species in riverene sediment are resuspended through tidal and storm action, they may be carried seaward into the surface layers of the stratified upper bay and out into shelf waters, but only in small numbers. As the number of methanogens increases in the particle-rich pycnocline, new taxa encountered for the first time in the pycnocline, may be selectively enriched in the sedimenting particles as they pass into the bottom water layer

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