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Characterization of free-living and attached bacteria in sediments colonized by *Hediste diversicolor*

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ABSTRACT: The dynamics of free-living and attached bacteria populations were studied in salt marsh sediments that were extensively colonized by the polychaete *Hediste* (*Nereis*) *diversicolor* (O. F. Müller). Bioturbation by *H. diversicolor* affected the density and community structure of free-living (FLB) and attached (AB) bacteria assemblages. Passage through the polychaete gut resulted in an 81 to 88% decrease of both bacterial groups and shift in favor of FLB in the hindgut (HG). In surficial sediments outside and inside the worm bed, attached bacteria represented 77 to 99% of the total bacterial densities. Although FLB were minor in terms of biomass, their densities strongly increased in January and May and peaked from 9.6 to 16.2×10^8 cells g^{-1}. AB peaked in August and October with densities from 205.8 to 283.6×10^8 cells g^{-1}. AB densities were significantly higher in burrow wall sediment than in surrounding sediment. The density of FLB and AB was reduced in surficial sediment inside the worm bed compared to sediments not colonized by *H. diversicolor.* Community fingerprints of environmental 5S rRNA showed that there was no significant difference between the structure of the 2 bacterial assemblages. However, in August 1995, the 2 assemblages were highly divergent in the sediment outside the worm bed. The structure of FLB and AB from inside the worm bed was significantly different from the structure of bacterial assemblages outside the sediments colonized by *H. diversicolor*.

KEY WORDS: Salt marsh · Sediment · Bioturbation · Polychaete · Bacteria · 5S rRNA

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INTRODUCTION

During the past decades, research on benthic food webs has focused on the relative importance of detritus versus bacteria in the diet of benthic macrofauna (Lopez & Levinton 1987), while the effect of macrofauna on the bacterial community has received less attention (Kemp 1990). However, some results indicate that deposit-feeders, such as polychaetes, can either control or stimulate bacterial biomass and activity directly by predation or indirectly by bioturbation (Moriarty et al. 1985, Grossmann & Reichardt 1991, Reichardt et al. 1991). Studies analyzing how marine deposit-feeders affect microbial variables focused on diverse species of crustaceas, holothurias and molluscs. Few reports deal with polychaetes (Aller & Yingst 1985, Duchene et al. 1988, Reichardt 1988, Plante et al. 1989, Grossmann & Reichardt 1991, Plante & Jumars 1993, Steward et al. 1996, Plante & Shriver 1998, Phillips & Lovell 1999). Among polychaetes, data dealing with the deposit-feeder and borrowing *Hediste* (*Nereis*) *diversicolor* (O.F. Müller) are scarce. However, this polychaete causes substantial changes in oxygenation and redox potential of surficial and burrow-lining sediments that may have a significant effect on the bacterial assemblages (Esselink & Zwarts 1989, Gilbert et al. 1994). For instance, denitrification rates increased in surface sediments with *H. diversicolor* colonies and activity of chemolithotrophic bacteria was stimulated in burrows (Gilbert et al. 1994). Lucas &

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Bertru (1997) demonstrated that >80% of ingested sediment bacteria disappeared in the *H. diversicolor* midgut (MG) via bacteriolysis. Lysis in the MG and regrowth in the hindgut (HG) of diverse bacterial strains may occur at different rates, thus altering the bacterial community composition of feces and of surrounding sediments (Plante & Shriver 1998). However, field studies supporting this hypothesis are needed.

A few studies have examined the impacts of deposit-feeding infauna on microbial community structure using either cultivation techniques (Findlay & White 1983, Duchêne et al. 1988) or analysis of phospholipid fatty acid (PLFA) profiles (Dobbs & Guckert 1988a,b, Steward et al. 1996, Phillips & Lovell 1999, Bird et al. 2000). Cultivation techniques have drawbacks because isolates typically represent less than 1% of natural bacterial communities (Amann et al. 1995). The use of cultivation-independent techniques based on nucleic acid and/or PLFA analyses avoids this bias. However, many of these techniques are time consuming, not quantitative, and sometimes data complexity constitutes substantial challenges for analysis. There is a clear need for methods that can be used to rapidly examine microbial community structure. One simple approach is to separate bacteria into easily recognizable groups such as free-living (FLB) and attached bacteria (AB) (Pedros-Alio 1989). Using molecular techniques, differences in the taxonomic structure between FLB and AB assemblages have been demonstrated in the water column (Delong et al. 1993, Acinas et al. 1999, Crump et al. 1999, Selje & Simon 2003). PCR-based techniques, such as cloning and DGGE profiles, allow the analysis of dominant species in the total community. While avoiding PCR biases, 5S rRNA profiles directly address the distribution and relative abundance of dominant active members of bacterial community (Höfle 1992), since the amount of RNA is well correlated with the growth rate of bacteria (Kemp et al. 1993). Closely related organisms usually have 5S rRNA of the same size, which allows a resolution at the genus level (Höfle 1992). 5S rRNA analysis has been successfully applied to study the community dynamics of bacterioplankton (Höfle 1992, Bidle & Fletcher 1995, Höfle et al. 1999).

Developing methods to understand how the polychaete *Hediste diversicolor* influences bacterial community structure is important as this worm may affect the activity and occurrence of key functional groups within benthic ecosystems. Bacteria in marine sediments control most biogeochemical cycles and benthic food web dynamics (Azam et al. 1983). To understand how lysis and regrowth in the deposit-feeder digestive's tract and bioturbation, resulting from burrowing activity, may affect the structure of bacterial communities, we studied the temporal and spatial distribution and structure of FLB and AB in gut contents of *H. diversicolor* and in bioturbated sediments. The taxonomic structure of active members of these 2 bacterial groups was directly analyzed using 5S rRNA profiles.

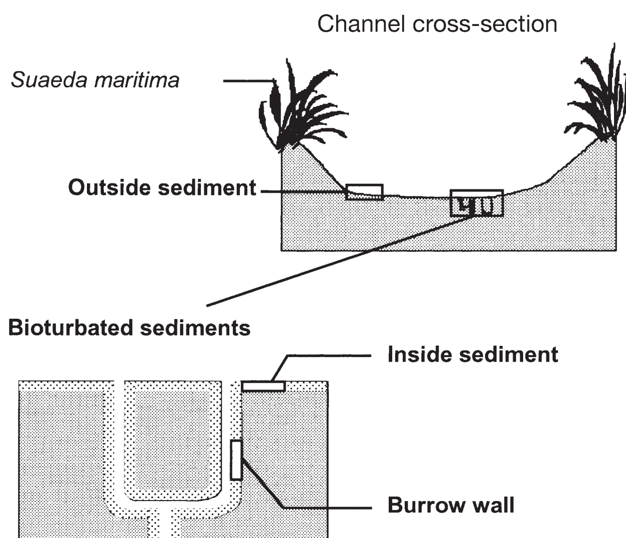

Fig. 1. *Hediste diversicolor.* Location of outside, inside and wall sediments in the drainage channel

MATERIALS AND METHODS

Study site. The salt marsh was situated in the Bay of Mont St Michel, France (48° 40′ N, 1° 40′ W). Adult worms (n = 10) were collected on October 9, 1995 by digging in a tidal channel. Digestive tracts of 5 worms were divided into MG and HG, and dissected as in Lucas & Bertru (1997). Five *Hediste diversicolor* were also left to fast for 12 h in order for their feces to be collected. Gut samples and feces from 5 worms were pooled in order to count bacteria in ingested sediments.

Sediment samples were collected in the tidal channel on October 9, 1995 (O95) and January 17, May 23, August 27 and October 4, 1996 (J96, M96, A96, O96, respectively). Surficial sediments were sampled (6 g at 0 to 1 cm deep): (1) on the border of the channel, outside the *Hediste diversicolor* bed and called 'Outside' (i.e. not bioturbated by *H. diversicolor*); and (2) inside the worm bed and called 'Inside' (Fig. 1). Burrow walls were sampled (1 g) by digging up a block of sediment at low tide and scrapping the sediment from the burrow wall (0 to 3 mm) using a sterile spatula; these sediments were called 'Wall' (Fig. 1). Samples were placed in sterile bags on ice for transport to the laboratory.

Bacterial counts. FLB and AB were extracted from 3 replicate subsamples of the homogenized gut, feces and sediment samples. FLB were extracted from samples (complete sample for the gut and feces, 1 g for out-

side and inside sediments and 0.3 g for wall sediment) by washing with 10 ml of sterile artificial seawater. After agitation for 10 min at 150 rpm, the sediment slurry was centrifuged 15 min at 500 g. Supernatants were kept for direct counts and RNA extraction of FLB. AB were extracted from sediment pellets according to Lucas et al. (1996). Briefly, the pellets were sonicated for 10 min with 200 mg of Chelex 100 (Bio-Rad) and 10 ml of 0.1% sodium deoxycholate and 2.5% PEG 6000 solution. After centrifugation, supernatants were kept for direct counts and RNA extraction of AB. Bacteria in supernatants were stained with acridine orange and were counted using an epifluorescence microscope (Hobbie et al. 1977). Counts were reported as average number per g of dry sediment (DW). Statistical comparison of densities was conducted using multivariate analysis of variance and Tukey's multiple comparison tests (Statgraphics, Manugistics).

5S rRNA extraction and labeling. The small amount of sample we collected from the burrow wall and gut contents did not allow us to obtain enough cells for RNA extraction. As a consequence, RNA was only extracted from outside and inside samples. FLB and AB were collected from supernatants by centrifugation for 10 min at 10 000 × *g*. RNA was extracted from bacterial biomass according to Tsai et al. (1991), slightly modified by adding 20% polyvinylpolypyrolidone at the beginning of the procedure. Crude extracts were then purified using Qiagen-100 columns (Qiagen SA). One to 4 µg of total RNA was mixed with 10 µl of buffered T4 RNA-ligase (0.5 U μl^{-1}, Pharmacia) and were 3′-end labeled with 10 µCi (0.37 MBq) of [5′-32P]-cytidine 3′,5′ diphosphate (specific activity: 3000 Ci $mmol^{-1}$, Amersham), according to England et al. (1980). Samples were kept overnight at –20°C, then they were precipitated with ethanol.

Separation of 5S rRNA and quantitative analysis. Radioactively labeled 5S rRNA was separated using denaturing high power electrophoresis on a 10% polyacrylamide gel (Sequigel electrophoresis: 380 × 800 × 0.4 mm, Bio-Rad) according to Höfle (1992). Electrophoresis conditions included stepwise increase over 5 h from 100 to 250 W. After electrophoresis, the gel was fixed in 1% acetic acid for 30 min, washed with distilled water and air dried. 5S rRNA profiles were revealed by exposure to X-ray film for 30 min to several hours. Some samples failed to give band profiles: outside sediment from October 1996 and inside sediment from August 1996 for FLB assemblages; outside sediment from January 1996 for AB.

5S rRNA profiles were standardized and analyzed using Gelcompar (Applied Maths, BVBA) computer software. The relative number of nucleotides and the intensity of each 5S rRNA band was estimated by reference to standards (*Escherichia coli* 5S rRNA) and the relative intensity of each band per sample was calculated as percentage of total band intensity (area under the densitometric profile). For diversity and similarity analyses, only bands with relative intensity >4% were used. Complete profiles are given in Table 1. Each band of different size is considered as an operative taxonomic unit (OTU). Specific diversity was estimated using the Shannon index (Shannon & Weaver 1963):

$$H' = \sum_{s}^{i=1} p_i \times \log_2 p_i \qquad (p_i = n_i / N)$$

where N is the total intensity of 5S rRNA per sample and n_i the intensity for each band (Lucas 1997, Höfle et al. 1999). The Shannon index increases with the number of species and is balanced by the frequency of each species (Shannon & Weaver 1963).

Table 1. Relative intensity of 5S rRNA bands (percentage of the total amount of 5S rRNA sample) for free-living (FLB) and attached bacteria (AB) in outside and inside sediments, in October 1995 (O95) and January (J96), May (M96), August (A96) and October (O96) 1996. Each band represents an operational taxonomic unit (OTU)

Sediment	Bacteria	Date	121	120	119	118	117	116	115	114	113	112	111	109
Outside	FLB	O95	40	10			40				5	5		
		M96		56		28	15					1	10	
		A96		8	18	23	44							
	AB	O95				40	47							
		M96				54	46							
		A96	29		19	23	30							
		O96				39	61							
Inside	FLB	J96		9			58	33						
		M96	29			64								8
		O96	36	42		22								
	AB	M96	38		35	16				1	3	1	5	
		A96	18	16	18	48								
		O96	41	52		7								

Similarity between samples was calculated using the Sorensen index (Cs; Sorensen 1948), where j is the number of common bands, and a and b the number of bands in the samples A and B: $Cs = 2j/(a+b)$. A Sorensen value of 1 is expected for identical samples and 0 for dissimilar samples. The Sorensen matrix was compared to binary matrices for the sediment type, the bacterial assemblage and the date. Using the software Fstat 2.9.3.1 (Goudet 1995), partial correlation of the matrices was performed with the Mantel test and a Monte Carlo procedure (5000 permutations) was used to determine statistical significance (Manly 1991).

RESULTS

Bacterial densities in the digestive tract of *Hediste diversicolor*

As shown in Fig. 2A, FLB densities from inside sediment (4.2×10^8 cells g^{-1}) were 27 times lower than AB densities (115.1×10^8 cells g^{-1}). For both bacterial groups, densities were significantly ($n = 24$, $p < 0.01$) lower in the MG samples versus inside sediment (Table 2). Selection and/or digestion of bacteria resulted in a decrease of 81% for FLB densities and 88% for AB densities in MG (Fig. 2A, Table 3). When comparing HG versus MG, results differed between FLB and AB, with a significant ($n = 24$, $p < 0.01$) increase (55%) for FLB and decrease (95%) for AB (Tables 2 & 3).

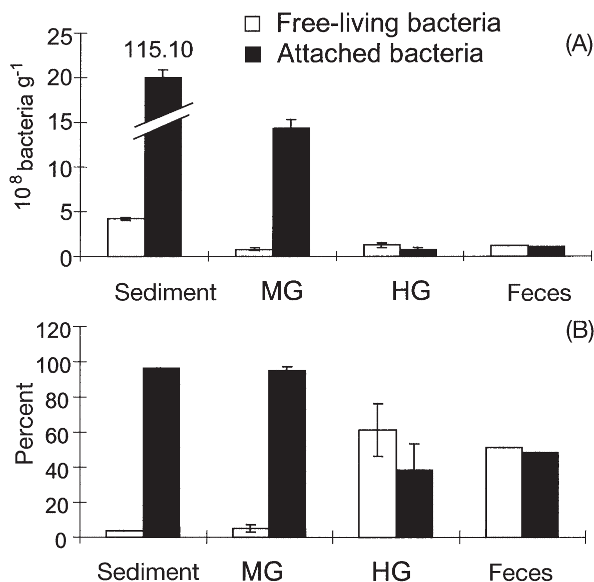

Fig. 2. (A) Bacterial density (10^8 cells g^{-1} DW) and (B) relative contribution in percent of free-living (FLB) and attached (AB) bacteria in inside sediment, midgut (MG) and hindgut (HG) content, and feces of *Hediste diversicolor*. Error bars denote SD (n = 3)

Table 2. Variance analysis and Tukey's test ($n = 24$, $p < 0.01$) of densities and percent of free-living (FLB) and attached (AB) bacteria in inside sediment, midgut (MG) and hindgut (HG) contents, and feces of *Hediste diversicolor*. No significant difference was found between the underlined groups

	Bacteria	Sediment
Density	AB	HG Feces < MG < Sediment
	FLB	HG < Feces MG < Sediment
Percent	AB	HG Feces < MG Sediment
	FLB	Sediment MG < Feces HG

In feces, bacterial abundances were not significantly different from densities in HG samples (Table 2). The increase of AB (42.3%) in the feces was not significant ($n = 24$, $p > 0.05$) due to high SD (Tables 2 & 3).

Percent of FLB and AB in digestive tract of *Hediste diversicolor*

Attached bacteria dominated (94 to 97%) the bacterial community in inside sediment and the MG. However, the relative proportions of FLB and AB shifted in the HG and feces, with AB representing only 40 to 48% of the bacterial community (Fig. 2B). FLB content was significantly ($n = 24$, $p < 0.01$) higher in the feces and the HG, while AB densities were significantly ($n = 24$, $p < 0.01$) higher in inside sediment and MG than in HG and feces (Table 2). There was no significant difference between HG and the feces for both bacterial fractions (Table 2).

Bacterial densities in outside and inside sediments

Bacterial densities from outside, inside and wall sediments are reported in Fig. 3. FLB densities ranged from 0.7 to 16.2×10^8 cells g^{-1} (Fig. 3A) and AB densities ranged from 36.7 to 284.2×10^8 cells g^{-1} (Fig. 3B).

Seasonal variations

FLB concentrations increased in January 1996 (16.2×10^8 cells g^{-1}) and May 1996 (9.6×10^8 cells g^{-1}) in the inside sediments. In outside sediments, FLB densities peaked in May 1996 (11.9×10^8 cells g^{-1}) and for the wall sediments, the FLB abundances were higher in August 1996 (Fig. 3A). FLB densities in May 1996 were significantly higher than in October 1995 and 1996, and August 1996 (Table 4). Percentage of FLB significantly increased in January and May 1996 and was highest in May 1996 ($n = 42$, $p < 0.01$).

Table 3. Change in proportion of free-living (FLB) and attached (AB) bacteria densities along the digestive tract of *Hediste diversicolor* (difference between average density of Sample 1 in comparison to Sample 2)

Difference between 2 samples	FLB	AB
Midgut content vs inside sediment	–80.7	–87.5
Hindgut vs midgut content	55.0	–94.5
Feces vs hindgut content	–4.8	42.3

AB densities increased in October 1995 and August 1996 in the 3 sediments, with maximum densities ranging from 76.5 to 283.6 × 10^8 cells g^{-1} (Fig. 3B). The lowest densities were reported in January 1996 with 39.6 × 10^8 cells g^{-1} in outside sediment, 36.7 × 10^8 cells g^{-1} in inside sediment and 49.9 × 10^8 cells g^{-1} in burrow walls. AB increase in October 1995 and August 1996 was significant (Table 4). The percent of AB was significantly higher in October 1995, August 1996 and October 1996 (n = 42, p < 0.01; Table 4).

Differences between sediments

In outside sediments, the FLB densities ranged from 2.3 to 16.2 × 10^8 cells g^{-1} (Fig. 3A). Densities were lower in inside sediments (0.3 to 11.9 × 10^8 cells g^{-1}) and burrow walls (0.3 to 8.3 × 10^8 cells g^{-1}). FLB densities were significantly higher in outside sediments than inside and wall sediments (Table 4). There was no significant difference between the FLB abundances from inside and wall sediments. However, the percentage of FLB was significantly lower in wall sediments than inside sediments (Table 4).

AB densities were the highest in burrow walls (49.9 to 284.2 × 10^8 cells g^{-1}) and outside sediments (39.6 to 246.0 × 10^8 cells g^{-1}). AB abundances were lower in inside sediments, ranging from 36.7 to 115.1 × 10^8 cells g^{-1} (Fig. 3B). AB densities from inside sediments were significantly lower than from wall and outside sediments (Table 4). The percentage of AB was significantly higher in the burrow walls and significantly lower in the outside sediments (Table 4).

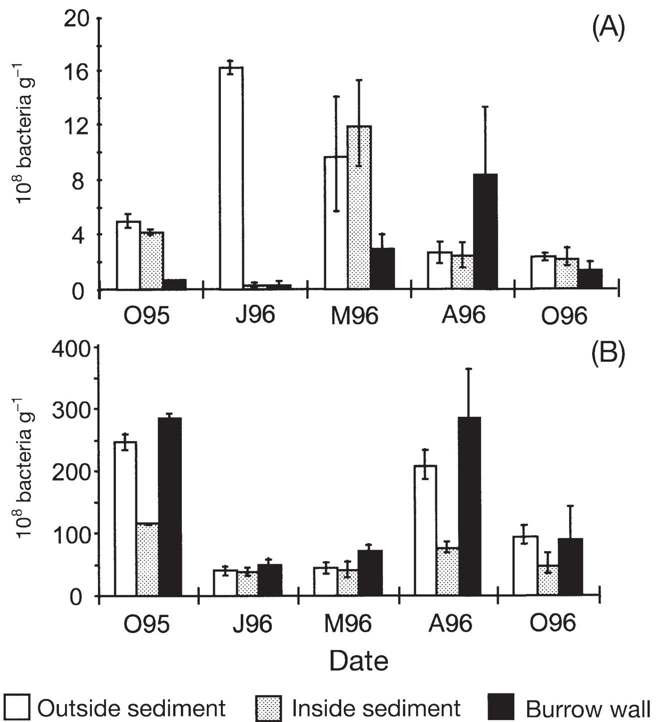

Fig. 3. (A) Number of free-living (FLB) and (B) attached (AB) bacteria in outside, inside and burrow wall sediment, according to the sampling date. Error bars denote SD (n = 3)

Analysis of 5S rRNA profiles from outside and inside sediments

Fig. 4 shows the contribution of bands with a relative intensity >4 % to each profile. FLB and AB were characterized by a low number of OTUs as indicated by the number of bands of different size (in average 2 to 5 bands per sample; Fig. 4). Analysis of 5S rRNA profiles are limited to the active part of the community, which may explain the low number of OTUs observed in our sediment samples. In all the samples, a total of 12 different bands was recognized, ranging from 109 to 121 nucleotides (nt) in size. Major bands contained 115 to 121 nt (Fig. 4). The Shannon index of 5S rRNA profiles varied from 0.96 to 1.98. As reported in Table 5, the FLB assemblage had a higher average Shannon index (1.77 ± 0.05) than AB (1.32 ± 0.47) in the outside sediment. However, in the inside sediment, the average Shannon index was lower for FLB (1.34 ± 0.16) than for AB (1.60 ± 0.28). There was no significant difference between FLB and AB (ANOVA: $F_{1,12}$ = 0.27, p = 0.61), there was no significant difference between outside

Table 4. Variance analysis and Tukey's test (n = 42, p < 0.01) of densities and percent of free-living (FLB) and attached (AB) bacteria in outside, inside and burrow wall sediments. Underlined groups do not show significant differences

	Bacteria	Sediment	Season
Density	FLB	Wall Inside < Outside	O96 O95 A96 J96 M96
	AB	Inside < Outside Wall	J96 M96 O96 < A96 O95
Percent	FLB	Wall < Inside < Outside	O96 O95 A96 < J96 < M96
	AB	Outside < Inside < Wall	M96 < J96 < O96 A96 O95

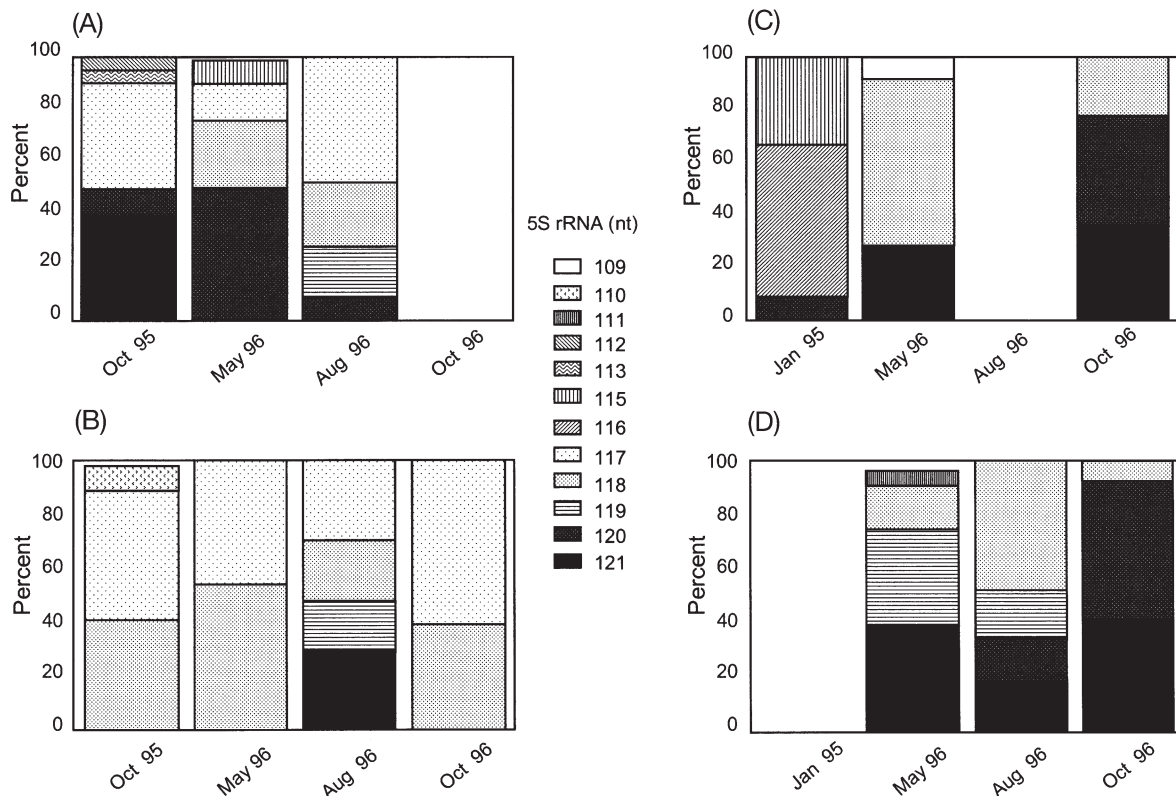

Fig. 4. 5S rRNA profiles for free-living and attached bacteria in (A,B) outside and (C,D) inside sediments, respectively. The relative contribution of each band per sample is expressed as a percent of the total amount of 5S rRNA in the sample (bands <4 % are not represented). The size of the bands is expressed in nucleotides (nt)

and inside sediments ($F_{1,12}$ = 0.16, p = 0.69). The interaction between the bacterial fraction and the sediment type was not significant ($F_{3,12}$ = 4.00, p = 0.08). Given the low number of samples, the validity of the statistical tests has to be taken with caution.

The structure of FLB and AB assemblages was compared using the Sorensen index (Table 6). To simplify the analysis, we used the Mantel test. However, these results have to be interpreted with caution because of the low number of samples being compared. Also, the Mantel test masked the dynamic aspect of the assemblages. For the structure of both FLB and AB, there was a significant difference between outside and inside sediments (Table 7). However, in August 1996, AB assemblages from inside and outside sediments shared similar structures (Sorensen index of 0.75; Table 6).

There was no significant correlation between the bacterial structure and the sampling date (Table 7). The structure of AB assemblages seemed more stable over the time period sampled than the FLB fraction, especially in outside sediment. In this sediment, the 117 and 118 bp OTUs dominated the AB community at each sampling date (Fig. 4). When comparing samples of the same sediment on different dates, AB Sorensen indices varied from 0.57 to 1.00, while FLB Sorensen indices ranged from 0.00 to 0.75 (Table 6).

The structure of FLB assemblages was not different from the structure of AB assemblages (Table 7). AB and FLB structure mostly differed in low molecular weight bands (109 to 116 nt), which appeared in January and May (Fig. 4, Table 1). In August and October, the FLB and AB assemblages were dominated by medium size bands (117 to 120 nt) or high molecular weight bands (121 nt). The Sorensen matrix

Table 5. Overall diversity, expressed as Shannon index of free-living (FLB) and attached (AB) bacteria in outside and inside sediments based on 5S rRNA community fingerprints. nd: not determined

Sediment	Bacteria	O95	J96	M96	A96	O96	Average ± SD
Outside	FLB	1.82	nd	1.73	1.75	nd	1.77 ± 0.05
	AB	1.35	nd	1.00	1.98	0.96	1.32 ± 1.47
Inside	FLB	nd	1.30	1.19	nd	1.54	1.34 ± 0.16
	AB	nd	nd	1.70	1.82	1.29	1.60 ± 0.28

Table 6. Sorensen index of free-living (FL) and attached (A) bacteria from outside (O) and inside (I) sediments in October 1995 (O95), January (J96), May (M96), August (A96) and October (O96) 1996

	OFLO95	OFLM96	OFLA96	OAO95	OAM96	OAA96	OAO96	IFLJ96	IFLM96	IFL096	IAM96	IAA96
OFLM96	0.44											
OFLA96	0.44	0.75										
OAO95	0.25	0.86	0.57									
OAM96	0.29	0.67	0.67	0.80								
OAA96	0.44	0.5	0.75	0.57	0.67							
OAO96	0.29	0.67	0.67	0.80	1.00	0.67						
IFLJ96	0.25	0.29	0.29	0.00	0.00	0.00	0.00					
IFLM96	0.00	0.29	0.29	0.33	0.40	0.57	0.40	0.00				
IFL096	0.50	0.57	0.57	0.33	0.40	0.57	0.40	0.33	0.67			
IAM96	0.22	0.50	0.50	0.29	0.33	0.75	0.33	0.00	0.57	0.57		
IAA96	0.44	0.50	0.75	0.29	0.33	0.75	0.33	0.29	0.57	0.86	0.75	
IAO96	0.50	0.57	0.57	0.29	0.40	0.57	0.40	0.33	0.67	1.00	0.57	0.86

showed that FLB and AB similarity varied with the sampling period, for example in October 1995, FLB and AB structure were very dissimilar (Table 6). Sorensen indices comparing FLB to AB structures ranged from 0.25 to 0.75 in outside sediments and 0.57 to 1.00 in inside sediments.

DISCUSSION

In the Bay of Mont St Michel, the polychaete *Hediste diversicolor* is among the most abundant macroinvertebrates in the salt marsh drainage channels (field observation). This worm has various feeding strategies, including deposit-feeding (Esselink & Zwarts 1989). As a deposit-feeder, *H. diversicolor* may have a great impact on the microbial community by grazing sediment bacteria.

Most sediment bacteria are attached to sediment grains or other large particles (Dye 1983, Ellery & Schleyer 1984, Ozawa & Yamaguchi 1986), which makes them more available to macrofaunal grazers (Plante & Shriver 1998). In our study, we found that AB represented 76.9 to 99.2% of the total bacterial community in sediment inside the worm bed. In the digestive tract of *Hediste diversicolor*, we observed that 81% of FLB and 88% of AB were removed in the MG in comparison to surficial sediment. This finding is in agreement with previous studies reporting removal of more than 50% of total bacteria with each passage through the gut of various polychaetes (Cammen 1980, Duchêne et al. 1988, Grossmann & Reichardt 1991, Lucas & Bertru 1997). Our data also allowed a differentiation between digestive and post-digestive processes. The number of FLB showed a 55% increase in the HG compared to the MG, while AB continued to decrease. As a result, the prevalent bacterial fraction in the HG and feces shifted to FLB.

The variation of bacterial densities through the gut may have different reasons: (1) sediment sorting; (2) digestion; and (3) bacterial growth and contribution by the gut flora (Dobbs & Guckert 1988b, Plante et al. 1989). Sediment sorting may have played a significant role in altering the bacterial community as *Hediste diversicolor* enriches its intake in organic matter versus inorganic sediment particles (Gunnarsson et al. 1999). However, such a process will enrich the bacterial community in the animal gut (Lopez & Levinton 1987) and not decrease the bacterial densities. Bacterial digestion can certainly explain the decrease we observed since high bacteriolytic activities were measured in the MG of *H. diversicolor* (Lucas & Bertru 1997). A shift in the relative proportion of FLB and AB in the *H. diversicolor* HG can be explained by a rapid growth of FLB using digestion products (Plante et al. 1989) or by AB becoming free after the action of digestive enzymes. Differential fate of FLB versus AB in the gut could be explained by taxonomic differences, although there is no clear consensus on this matter in the literature (Hollibaugh et al. 2000). Differential lysis of diverse bacterial taxa has been reported previously. Plante & Shriver (1998) demonstrated *in vitro* that Gram-positive bacteria are more resistant to bacteriolysis by *Arenicola marina* MG fluids, and some studies have demonstrated *in situ* changes of bacterial community

Table 7. Partial correlation (Mantel test, n = 78) between the Sorensen matrix and bacterial, spatial and temporal data. Values in **bold** represent significant correlation ($p < 0.05$)

Factor	R	p
Sediment	**0.426**	**0.010**
Sampling date	0.110	0.238
Bacterial assemblage	0.023	0.544
Bacterial density	0.058	0.777

structure between ingested sediment, guts and feces (Duchêne et al. 1988, Dobbs & Guckert 1988b).

The changes in bacterial community structure that we documented in the *Hediste diversicolor* digestive tract may also affect the bacterial diversity in surrounding sediments. The effect depends on the abundance, feeding rate and digestive efficiency of deposit-feeders, relative to the bacterial growth rates (Plante & Shriver 1998). Biotic processes, such as irrigation activity, material translocation and mucus secretion by the polychaete, as well as abiotic processes, such as wave action, sediment reworking, organic matter deposition, groundwater seeping, seasonal variation of sediment pH, temperature and moisture, can also overshadow the effect of gut processing.

In our study, the most pronounced impact of *Hediste diversicolor* on the sediment bacterial community appeared in the burrow wall. The proportions and densities of AB in the lining of *H. diversicolor* were significantly higher than in outside and inside sediments. The dominance of AB in the burrow wall was probably due to the adhesive capacities of the mucus that cover its body and the burrow walls. Moreover, mucus secretion and excretion of urea and ammonium by the worm may also stimulate the growth and activity of bacteria, together with its irrigation activity (Reichardt 1988). Investigation of burrow microbial community structure was complicated by the small amount of material sampled. However, the 5S rRNA profiles that we obtained from total RNA extraction (not divided into FLB and AB) showed that the burrow wall community was stable over time and different from assemblages in outside and inside sediments (Lucas 1997). In terms of physico-chemical characteristics, infaunal burrow walls are fairly stable environments compared to the more frequently disturbed sediment/water interface (Steward et al. 1996). This stability over time allows for the development of complex microbial biofilms (Steward et al. 1996, Phillips & Lovell 1999). It leads to the formation of microbial communities fundamentally different from the nearby surface and subsurface sediments, with substantial bacterial diversity and high bacterial densities and growth rates (Steward et al. 1996, Dobbs & Guckert 1988a).

In surficial sediments, disturbance due to *Hediste diversicolor* burrowing and movement, as well as sediment mixing by physical processes (tidal or wave action, ripple migration) could have major impacts on densities and taxonomic structure of FLB and AB fractions. These processes may activate or stimulate the bacteria that would otherwise be slow growing or dormant (Findlay et al. 1990). In our study, FLB and AB densities were significantly lower in inside sediment than in outside sediment. We found it difficult to separate *H. diversicolor* activity or seasonal changes versus tidal flow within the channel or other disturbance to explain the changes in bacterial densities. Grain size analysis of outside and inside samples did not show any significant differences (data not shown). However, the high densities of bacteria in the undisturbed creek bank sediments may be explained by groundwater seepage, which is likely responsible for the 2 to 3 times higher carbon and nitrogen concentrations measured in the groundwater of the channel border compared to the channel water (Troccaz 1996). These nutrient rich conditions surely contributed to the difference in inside sediment situated in the middle of the channel.

FLB and AB densities in outside and inside sediments did not covary, suggesting that these 2 fractions responded to different environmental factors. The structure of the AB assemblages in the channel sediment seemed more stable throughout the year than the FLB structure. Attachment to particles may provide a more stable environment and protection against protozoan and meiofaunal grazing. Moreover, biofilms that cover surfaces also provide the opportunity for developing stable mutualistic interactions among the microbiota.

There was no taxonomic difference between the 2 fractions in the channel sediments. Other studies using fingerprinting methods (5S rRNA profiles and 16S rDNA DGGE profiles, respectively) also reported that FLB and AB assemblages in estuarine waters were similar (Bidle & Fletcher 1995, Hollibaugh et al. 2000, Selje & Simon 2003). These 2 assemblages of bacteria should be considered as interacting entities. Many exchanges can occur between these 2 groups via adsorption and desorption phenomena (Bright & Fletcher 1983, Karner & Herndl 1992). However, several studies using cloning methods suggest high taxonomic differences between FLB and AB assemblages (Delong et al. 1993, Acinas et al. 1999, Crump et al. 1999). The discrepancy between these reports may come from methodological biases, resulting in the analysis of different subsets of the total bacterial community. Firstly, these studies often used different methods to separate FLB from AB (Selje & Simon 2003). Secondly, fingerprint bands of similar position may represent completely different clones. Other important factors in determining the relative composition of AB and FLB assemblages may be the composition of the particle organic matter (Hollibaugh et al. 2000) and/or the ratio of organic and inorganic material in the sample (Selje & Simon 2003). The huge variation in similarity indices between FLB and AB assemblages that we found according to the sampling date may also be explained by variation in the quality of the organic matter.

This study showed that in sediment, FLB and AB can also be considered as interacting populations that have

different dynamics in response to the presence of *Hediste diversicolor* and seasonal variation. Separation of FLB and AB was useful in studying the relationship between sediment bacteria and macrofauna. However, further studies will help to elucidate the relative importance of abiotic versus biotic effects on spatial and temporal distribution of bacteria and the taxonomic structure of the sediment bacterial community. Estimation of bacterial production and rate of sediment turn-over due to *H. diversicolor* feeding and defecation will also be useful for knowing how important these processes are in regulating bacterial densities and structure.

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