specific competition to stonefly growth and fecundity. Oecologia **88:** 277–288.

———, AND M. A. PENTON. 1990. Effects of enclosures on stream microhabitat and invertebrate community structure. J. N. Am. Benthol. Soc. **9:** 249–261.

———, B. W. TAYLOR, A. R. MCINTOSH, M. A. MCPEEK, AND D. A. LYTLE. 2001. Variation in mayfly size at metamorphosis as a developmental response to fish predation. Ecology **82:** 740–757.

PHILLIPS, B. F., AND A. B. SCOLARO. 1980. An electrofishing apparatus for sampling sublittoral benthic marine habitats. J. Exp. Mar. Biol. Ecol. **47:** 69–75.

PRINGLE, C. M., AND G. A. BLAKE. 1994. Quantitative effects of atyid shrimp (Decapoda: Atyidae) on the depositional environment in a tropical stream: Use of electricity for experimental exclusion. Can. J. Fish. Aquat. Sci. **51:** 1443–1450.

RABENI, C. F., K. J. COLLIER, S. M. PARKYN, AND B. J. HICKS. 1997. Evaluating techniques for sampling stream crayfish (*Paranephrops planifrons*). N. Z. J. Mar. Freshw. Res. **31:** 693–700.

RADER, R. B. 1997. A functional classification of the drift: Traits that influence invertebrate availability to salmonids. Can. J. Fish. Aquat. Sci. **54:** 1211–1234.

RESETARITS, W. J., AND J. BERNARDO [EDS.]. 1998. Experimental ecology. Oxford Univ. Press.

SCHINDLER, D. W. 1990. Experimental perturbations of whole lakes as tests of hypotheses concerning ecosystem structure and function. Oikos **57:** 25–41.

SIH, A., AND D. E. WOOSTER. 1994. Prey behaviour, prey dispersal, and predator impacts on stream prey. Ecology **75:** 1199–1207.

STEINMAN, A. D. 1996. Effects of grazers on stream freshwater benthic algae, p. 341–373. *In* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe [eds.], Algal ecology: freshwater benthic ecosystems. Academic Press.

STEVENSON, R. J. 1996. The stimulation and drag of current, p. 321–336. *In* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe [eds.], Algal ecology: freshwater benthic ecosystems. Academic Press.

STEWART-OATEN, A., J. R. BENCE, AND C. W. OSENBERG. 1992. Assessing effects of unreplicated perturbations: No simple solutions. Ecology **73:** 1396–1404.

TAYLOR, B. W., A. R. MCINTOSH, AND B. L. PECKARSKY. 2001. Sampling stream invertebrates using electroshocking techniques: Implications for basic and applied research. Can. J. Fish. Aquat. Sci. **58:** 1–9.

WALLACE, J. B., T. F. CUFFNEY, J. R. WEBSTER, G. J. LUGTHART, K. CHUNG, AND B. S. GOLDOWITZ. 1991. Export of fine organic particles from headwater streams: Effects of season, extreme discharges, and invertebrate manipulation. Limnol. Oceanogr. **36:** 670–682.

WOOTTON, J. T. 1997. Estimates and tests of per capita interaction strength: Diet, abundance, and impact of intertidally foraging birds. Ecol. Monogr. **67:** 45–64.

YASUNO, M., S. FUKUSHIMA, AND J. HASEGAWA. 1982. Changes in benthic fauna and flora after application of temephos to a stream on Mt. Tsukuba. Hydrobiologia **89:** 205–214.

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Quantification, base composition, and fate of extracellular DNA in marine sediments

Abstract—The discovery of high concentrations of DNA in marine sediments unaccounted for by living biomass suggests the presence of a large fraction of extracellular DNA, which might play an important role in gene transfer via natural transformation as well as in phosphorous biogeochemical cycling. But a universally accepted procedure for extracellular DNA extraction is not available yet. In this study, we developed a new nuclease-based procedure to extract extracellular DNA from marine sediments. Coastal sand and deep-sea mud samples were collected to test the efficiency of extracellular DNA removal from different sediment types. Extracellular DNA concentrations were quantified at six sediment depths, and changes in base composition were investigated to gather information on extracellular DNA fate. The extraction procedure was highly specific and only extracellular DNA was hydrolyzed after nuclease treatment. Hydrolyzable DNA accounted for <10 to $>70\%$ of the total DNA pool, suggesting that extracellular DNA can only be partially degraded. Base composition changed vertically with depth in the sediment as deoxycytidine content increased and deoxyadenosine decreased with increasing depth. Integrating our results for the top 15 cm of the sediment, we calculate that more than 50% of extracellular DNA was recalcitrant to enzymatic degradation. This finding might explain why DNA accumulates in surface sediments and suggests that DNA might play a nonnegligible role in P biogeochemical cycle.

Extracellular DNA is a ubiquitous component of both dissolved and particulate organic matter pools of freshwater, seawater, and benthic environments (Lorenz and Wackernagel 1994). Recent studies have shown that among aquatic systems, marine sediments from shallow depths down to the abyssal floor are characterized by high concentrations of extracellular DNA (Novitsky and Karl 1985; Danovaro et al. 1999; Dell'Anno et. al. 1998). The pool size of extracellular DNA in marine sediments is the result of complex interactions, including DNA inputs from the photic layer through particle sedimentation, autochthonous DNA production, and degradation or utilization or both by heterotrophic organisms (Dell'Anno et al. 1999). Extracellular DNA diagenesis in marine sediments is also influenced by DNA binding to complex refractory organic molecules, to inorganic particles, or to both, which might strongly reduce its enzymatic degradation (Nielsen et al. 1998). In this regard, Romanowski et al. (1991) showed that DNA adsorbed on sand and clay par-

ticles becomes 100- to 1,000-fold more resistant to DNase. Furthermore, when isolated from sediments, DNA is frequently complexed with humic substances, which stabilize DNA against nuclease degradation (Miller et al. 1999; Juniper et al. 2001). Consequently, the half-life of extracellular DNA in sediments appears to be much longer than in the water column (Lorenz and Wackernagel 1994).

Previous studies demonstrated that extracellular DNA is an important source of nitrogen and phosphorous for bacterioplankton metabolism (Turk et al. 1992; Kroer et al. 1994; Jørgensen and Jacobsen 1996) and a source of exogenous nucleotides, which are recycled by bacteria for the synthesis of new DNA (Paul et al. 1988, 1989). Moreover, extracellular DNA might participate in gene transfer via natural transformation of competent bacterial cells (Davison 1999). However, studies dealing with the horizontal transfer of recombinant DNA sequences or the biogeochemical pathways of extracellular DNA cycling have been almost completely restricted to the plankton domain (*see reviews in* Lorenz and Wackernagel 1994; Trevors 1996).

In this study, we developed a new nuclease-based procedure to extract extracellular DNA. This procedure mimics natural extracellular DNA hydrolysis by the addition of nucleases (Paul et al. 1988, 1989) and allows estimation of potential degradation, exploitation, or both by heterotrophic bacteria. In order to provide new insights into DNA diagenesis and fate in marine sediments, we also investigated changes in DNA base composition among sediment horizons. The aim of this study is to provide information on the potential role of sedimentary extracellular DNA in benthic trophodynamics and P biogeochemical cycling.

Materials and methods—Study area and sampling: In order to test the efficiency of the enzyme-based protocol for extracellular DNA removal from different sediment types, coastal sands and deep-sea muds were collected for this study. Sandy sediments (modal grain size ranging from 125 to 250 μm) were collected by hand coring (using Plexiglas tubes, 4.7 cm i.d.) in June 1998 at Falconara Beach (0.5 m deep; 43.6°N, 13.5°E, northern Adriatic, Mediterranean Sea). After collection, aliquots of the top 1 cm of sediment were immediately analyzed, while others were stored at −20°C and analyzed after 4 weeks. Deep-sea sediment samples were collected using a multicorer (Maxicorer, 9.0 cm i.d., depth penetration >20 cm) in September 1996, March 1998, and October 1998 in the Porcupine Abyssal Plain (PAP, 4,800 m deep; northeastern Atlantic Ocean, 48.50°N, 16.29°W). This area, characterized by strong seasonal and interannual variability in organic matter inputs (Dell'Anno et al. 1999), is assumed to represent the typical conditions of the deep-sea Atlantic Ocean. During each cruise, 4 to 10 cores were taken from four to seven different deployments. Upon recovery, all sediment cores were vertically sectioned into six layers (0–5, 10–20, 30–40, 50–60, 60–100, and 100–150 mm) and frozen at −20°C until analysis.

All solutions utilized in this study were prepared with MilliQ water and then autoclaved.

Extraction of extracellular DNA: The method presented here is based on time course experiments in which sediments were incubated with pools of commercial nucleases specifically selected for complete DNA hydrolysis. Enzyme characteristics and concentrations were chosen according to Amersham Pharmacia Biotech recommendations (http://www.amersham.co.uk/). We calculated (measuring absorbance change at 260 nm) that, at room temperature, enzyme concentrations utilized in these experiments were able to hydrolyze 20 μg ml^{-1} of double-stranded calf thymus DNA in solution in about 40 min.

Sediment samples of both sediment types were stirred at 150 rpm in 0.1 M Tris-HCl, 0.1 M NaCl, 1 mM $CaCl_2$, and $MgCl_2$ pH 7.5 (buffer : sediment ratio 2.5, v/wt). Sediment slurries were divided into aliquots of 1,945 μl, and replicate samples ($n = 3$) were added to 25 μl DNase I from bovine pancreas (1.9 U ml^{-1}), 10 μl nuclease P1 from *Pennicilium citrinum* (4.0 U ml^{-1}), 10 μl nuclease S1 from *Aspergillus orizae* (2.3 U ml^{-1}), and 10 μl esonuclease-3 from *Escherichia coli* (1.9 U ml^{-1}); another set of replicates was added to an equal volume of buffer, but without enzymes. Additional samples of sandy sediment, before the enzyme additions, were added to 20 μg of calf thymus DNA (final concentration of 10 μg ml^{-1}). Samples were incubated at room temperature for 0, 1, 2, 4, 6, and 24 h under gentle agitation. After incubation, all samples were chilled on ice and centrifuged at 2,000 × g for 5 min, and the supernatant, filtered through 0.2-μm Nuclepore filters (to remove suspended particles), was used to determine the amount of DNA released from the sediments as described below.

In order to assess the efficiency of extracellular DNA extraction by nuclease treatment, we compared this enzymatic procedure with the alkaline extraction protocol described by Ogram et al. (1987) in both sediment types. Ogram's procedure represents, to our knowledge, the only method available for extracellular DNA extraction from sediments. Sediment samples were mixed with 120 mM Na-phosphate buffer, pH 8.0, centrifuged at 2,000 × g for 5 min, and the supernatant was filtered through 0.2-μm Nuclepore filters. This procedure was repeated four times and the supernatant of each step was pooled and processed as described below.

We analyzed sediments immediately after sampling (fresh sandy sediments) and after 4 weeks at −20°C to evaluate differences potentially induced by storing.

Finally, in order to test the specificity of nuclease treatment in extracellular DNA removal, sediment samples treated with enzymes were mixed before stirring with *Escherichia coli* and *Tetraselmis* sp. cultures (collected during the exponential phase of growth) and compared with samples without bacterial or algal cultures. In both cases, we estimated that approximately 10 μg of bacterial or algal DNA were added. All samples were incubated for 2 h and subsequently analyzed as reported below.

Quantification and base composition of extracellular DNA: Supernatants obtained both from nuclease time course experiments and from the alkaline extraction procedure were dried under vacuum and analyzed fluorometrically using diaminobenzoic acid (DABA, Karl and Bailiff 1989). The fluorescence of hydrolyzed DNA was converted into concentrations using calibration curves obtained from standard solutions of calf thymus DNA (from 0.05 to 5.0 μg DNA

ml^{-1}). The amount of hydrolyzed DNA (HDNA) was obtained by difference between DNA concentrations (determined from the supernatant of enzyme-treated samples) and the supernatant of the control sample. This control supernatant contained <5% of the DNA released by nucleases in both sediment types. HDNA concentrations were normalized to sediment dry weight.

A subaliquot of the supernatant was also analyzed by reversed-phase high-performance liquid chromatography (HPLC) in order to identify and quantify the products of DNA hydrolysis (Tamaoka and Komagata 1984). In an additional experiment, we verified that standards of both commercial calf thymus DNA and deoxynucleotide monophosphates (Sigma Chemical Company) added to sediment samples were completely transformed into nucleosides after 2 h of incubation with the selected enzymes.

The HPLC system consisted of a Beckman 126-model pump, a Rheodyne model 7125 injector, a Beckman 166 model ultraviolet–visible (UV-Vis) detector, and a reversed-phase chromatographic C_{18} column (25 cm × 4.6 mm i.d., 5 μm particle size) equipped with a precolumn. The mobile phase consisted of 10 mM Na-phosphate, pH 7.0, pumped at a flow rate of 1 ml min^{-1}. Hydrolyzed DNA products were detected at 254 nm and identified by comparing their retention time with those of standard solutions of commercial deoxynucleosides spiked to sediment slurries (Sigma Chemical Company). The identified deoxynucleosides in the samples were then converted into concentrations from calibration curves obtained from the relationships between peak area of standards and their relative concentrations (Fig. 1A).

All chromatograms were integrated using Varian Star 5.5 software. When the peak shape of the sample didn't fit perfectly with the shape of the equivalent standard peak, a manual integration was performed. Retention times of standard nucleosides were 3.95, 5.0, 5.75, and 7.1 min for 2′-deoxycytidine (dC), 2′-deoxyguanosine (dG), 2′-deoxythymidine (dT), and 2′-deoxyadenosine (dA), respectively.

Total HDNA concentrations estimated using the fluorometric (DABA) assay displayed a significant relationship with total deoxynucleoside concentrations evaluated by HPLC analyses (Fig. 1B).

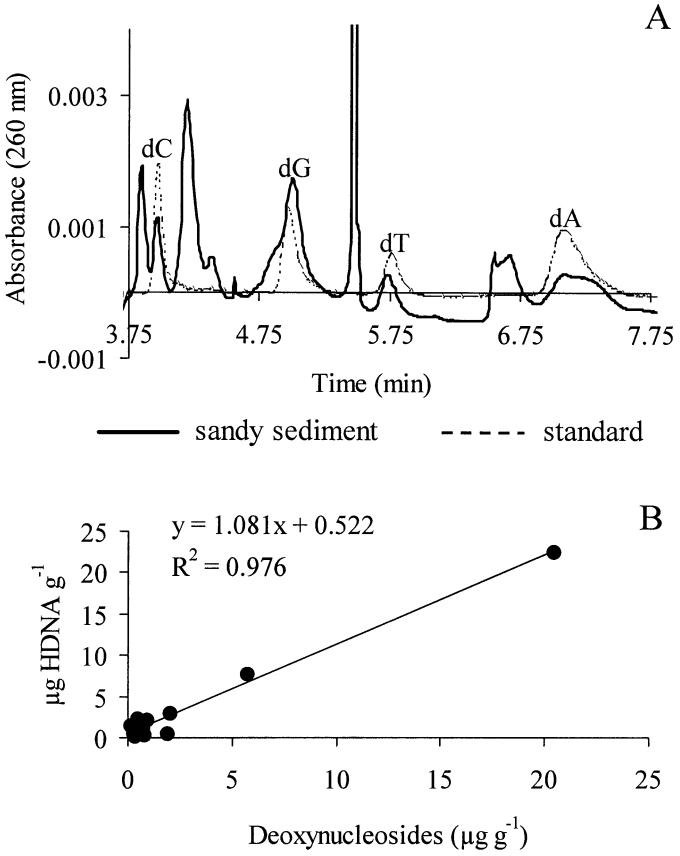

Fig. 1. (A) HPLC chromatograms of standards of deoxynucleosides and of DNA enzymatically hydrolyzed from sandy sediments. Reported are: 2′-deoxycytidine (dC), 2′-deoxyguanosine (dG), 2′-deoxythymidine (dT), and 2′-deoxyadenosine (dA). (B) Relationships between HDNA concentrations obtained from DABA assay and deoxynucleoside concentrations obtained from HPLC analyses (n = 12) in deep-sea sediments.

Extraction and quantification of total DNA concentrations: Data on total DNA concentrations in deep-sea sediments of the Porcupine Abyssal Plain have been summarized in Dell'Anno et al. (1999). Total DNA concentrations were determined in the same sediment cores used in this study by the procedure described by Dell'Anno et al. (1998). Briefly, 1 g of wet sediment (three replicates) was treated with 3.0 ml of 0.5 N perchloric acid, stirred for 3 min and sonicated three times for 1 min (at intervals of 30 s). DNA was hydrolyzed at 75°C for 30 min under continuous stirring and analyzed spectrophotometrically using diphenylamine. DNA concentrations were calculated using calibration curves obtained from standard solutions of calf thymus DNA. Data were normalized to sediment dry weight after desiccation (60°C, constant weight).

Data analysis: Differences between treatments were tested by the *t*-test after testing for homogeneity of variance. Analyses of variance (ANOVAs) followed by post hoc Tukey tests were carried out to test for spatial differences in HDNA concentrations in deep-sea sediment samples.

Results—Concentrations and base composition of hydrolyzed DNA in marine sediments: Time courses of DNA digestion of sandy and muddy sediment samples incubated with and without enzymes are illustrated in Fig. 2A,B. Supernatant fluorescence from treated samples rapidly increased in the first 2 h of incubation, whereas no significant changes were observed for control samples. Because no further increase of fluorescence was observed in enzymatically treated samples with increasing incubation time (i.e., up to 24 h), the optimal incubation time for DNA hydrolysis was standardized at 2 h. Also, calf thymus DNA standard added to sediments was completely hydrolyzed by nucleases in 2 h.

Independent of the sediment type, enzymatic treatments yielded significantly higher DNA concentrations compared to those obtained using the extraction procedure described by Ogram et al. (1987; Fig. 3A; *t*-test, $P < 0.01$ for both sediment types). Sample storage had no consequences on measured HDNA concentrations (16.9 ± 2.7 and 14.6 ± 3.1 μg g^{-1} for fresh and frozen samples, respectively; *t*-test, *P*

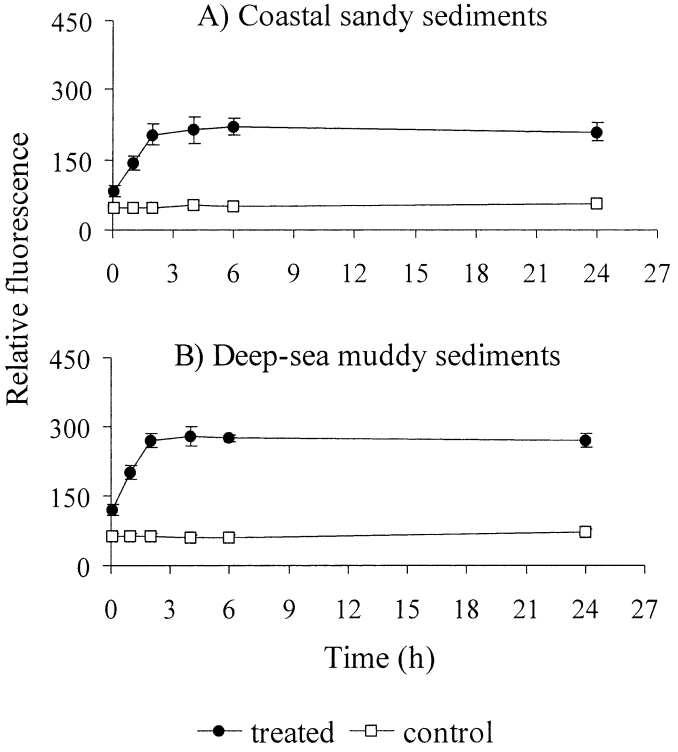

Fig. 2. Time course analysis of DNA release in treated and control (i.e., nonenzymatically treated) samples of (A) sandy and (B) muddy sediment samples. Data expressed as relative fluorescence units are referred to time course experiments carried out on the top 1 and 0.5 cm of sandy and muddy sediments, respectively. Standard deviations are reported (n = 3).

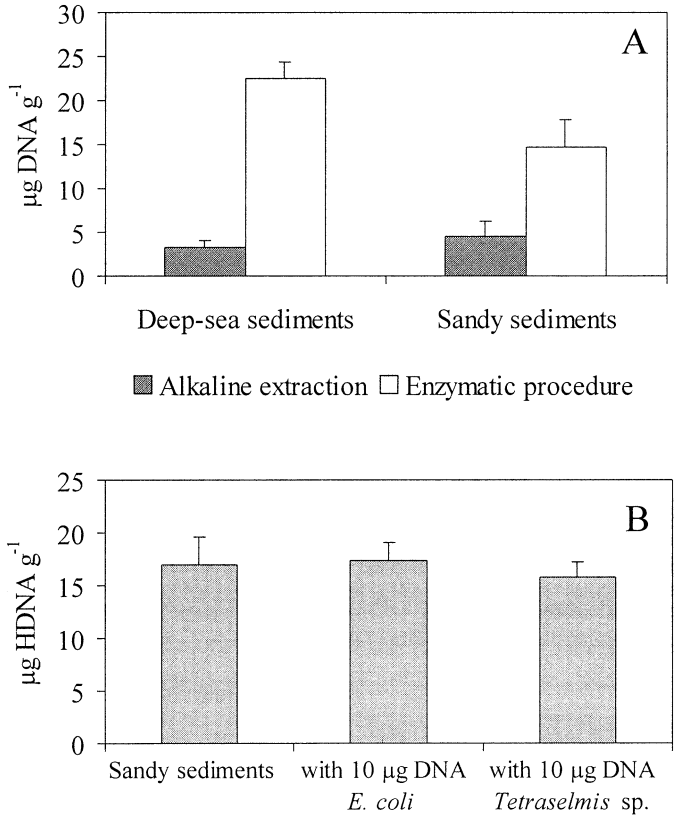

Fig. 3. (A) Comparison of DNA concentrations obtained by alkaline extraction (Ogram et al. 1987) and after enzymatic treatment in sandy (top 1 cm) and muddy (0.5 cm) sediments. Standard deviations are reported (n = 3). (B) Comparison between hydrolyzed DNA concentrations in sandy sediment samples with and without the addition of cell cultures. Standard deviations are reported (n = 3).

= 0.138). Nuclease treatment of natural sediments with and without added bacterial and algal cells showed no significant differences in HDNA concentrations (t-test, P = 0.292 and 0.308, respectively; Fig. 3B). In addition, HPLC analyses showed that enzymatic treatment did not result in the release of deoxynucleosides from intact microbial cells (Fig. 4).

HDNA concentrations in the top 5 mm of deep-sea sediments were highest in September 1996 (22.5 ± 1.9 μg g^{-1}) and lowest in March 1998 (7.8 ± 0.9 μg g^{-1}; Table 1). HDNA concentrations decreased significantly with depth in the sediment (ANOVA, $P < 0.01$), with highest values in the top 0–5 mm of sediment (16.5 ± 3.6 μg g^{-1}) and 40 times lower values in the deepest sediment horizon (i.e., 100–150-mm section, 0.4 ± 0.1 μg g^{-1}).

Base composition of hydrolyzed DNA in deep-sea sediments was characterized by the dominance of deoxycytidine (dC) and deoxyguanosine (dG) (on average, 35 ± 3% and 33 ± 2%, respectively, of the total hydrolyzable DNA pools) followed by deoxythymidine (dT, 22 ± 2%) and deoxyadenosine (dA, 10 ± 2%; Table 2). Analysis of the vertical profiles of DNA base composition highlighted clear differences between surface and deeper sediment horizons and a shift in base composition with depth in the sediment. Deoxycytidine increased with depth in the sediment core, whereas deoxyadenosine decreased (Table 2).

Discussion—Extracellular DNA in marine sediments: In the last decade, microbial ecologists, systematicists, and

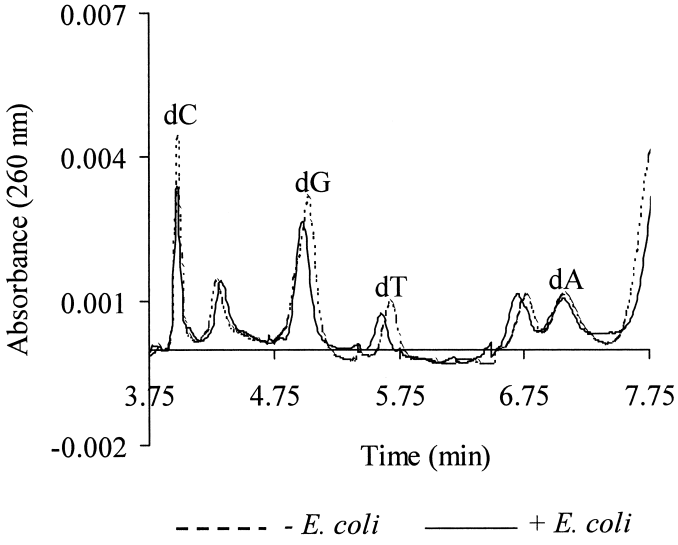

Fig. 4. HPLC chromatograms of nuclease-treated deep-sea sediment samples with and without the addition of intact *E. coli* cells. Reported are: 2′-deoxycytidine (dC), 2′-deoxyguanosine (dG), 2′-deoxythymidine (dT), and 2′-deoxyadenosine (dA).

Table 1. Vertical distribution of enzymatically hydrolyzed DNA concentrations (HDNA) and contribution (reported in parenthesis) of the enzymatically hydrolyzed DNA to the total DNA pools (expressed as percentage) in deep-sea sediments collected on three dates from the Porcupine Abyssal Plain. Standard deviations (±) are reported ($n = 6$).

Depth (mm)	HDNA (μg g^{-1})		
	September 1996	March 1998	October 1998
0–5	22.5±1.9(72±5)	7.8±0.9(63±10)	19.2±1.3(82±9)
10–20	2.9±0.5(27±7)	2.3±0.1(25±2)	5.8±1.9(57±14)
30–40	0.4±0.3(6±4)	1.1±0.9(16±13)	3.5±0.6(40±2)
50–60	2.1±0.6(19±5)	1.3±0.5(21±7)	1.7±0.2(29±2)
60–100	0.3±0.1(9±2)	1.5±0.4(27±7)	1.4±0.3(31±4)
100–150	0.2±0.1(6±1)	0.5±0.7(10±6)	0.4±0.2(12±4)

Table 2. Vertical profiles of base composition (expressed as molar percentage of the total deoxynucleosides) from DNA enzymatically hydrolyzed in deep-sea sediments collected in September 1996 and March 1998 from the Porcupine Abyssal Plain. Reported are 2′-deoxycytidine (dC); 2′-deoxyguanosine (dG); 2′-deoxythymidine (dT), and 2′-deoxyadenosine (dA). Standard deviations (±) are reported ($n = 6$).

Depth (mm)	September 1996				March 1998			
	dC	dG	dT	DA	dC	dG	DT	dA
0–5	34±1	32±2	15±1	19±1	15±1	44±2	25±1	16±1
10–20	27±2	37±6	29±3	7±1	35±10	30±7	21±2	14±8
30–40	47±3	18±1	22±1	13±1	29±2	30±8	23±4	18±5
50–60	43±2	30±3	18±1	9±1	32±1	24±3	41±5	3±2
60–100	45±3	30±2	18±1	7±4	31±8	49±3	14±4	6±1
100–150	37±5	37±1	23±3	3±2	48±1	35±11	15±8	2±1

population geneticists have become increasingly interested in methods for DNA isolation from sediments because such procedures promise to make the genomes of uncultured indigenous microorganisms available for molecular analysis (Head et al. 1998). The most utilized technique for DNA extraction from sediments is based on direct in situ lysis of the cells by means of physical (e.g., bead mill homogenization, ultrasonication, and freeze-thawing) and chemical procedures (e.g. using detergents such as SDS or Sarkosyl) or a combination of both (*see references in* Miller et al. 1999; Hurt et al. 2001). However, although in situ lysis has the potential to circumvent problems of biased representation of the microbial community, extracellular DNA is coextracted with nucleic acids from lysed cells, thus increasing the pool of templates and leading to an overestimation of the living biomass (Frostegard et al. 1999).

Isolation of extracellular DNA from sediments is a difficult task because treatments and procedures for the extraction of nucleic acids adsorbed to organic and inorganic particles may disrupt cells (Lorenz and Wackernagel 1994; Frostegard et al. 1999). Ogram et al. (1987) isolated extracellular DNA from freshwater sediments by washing samples four times with an alkaline Na-phosphate buffer. This procedure has been used repeatedly to avoid extracellular DNA contamination prior to direct in situ lysis (Atlas 1993; Purdy et al. 1996), but the use of this protocol for isolation of extracellular DNA has never been tested for extraction efficiency and intracellular DNA contamination.

In the present study, we used a new method for extracellular DNA extraction based on nuclease digestion of the sediment. In the past, an approach based on nuclease digestion has been applied only for testing the ability of purified DNA added on a pure sand or clay sedimentary matrix to degrade (Lorenz and Wackernagel 1987; Romanowski et al. 1991; Garko and Stewart 1994). This procedure is highly specific for extracellular DNA and is not affected by the presence of intracellular DNA because the addition of both eukaryotic and prokaryotic cells did not increase HDNA concentrations. Moreover, the possibility of contamination with intracellular DNA due to cell lysis during extraction can be rejected because the analysis of fresh and frozen sediment samples revealed identical HDNA concentrations. Another potential source of contamination could be nuclease-mediated degradation of viral DNA. However, Wommack and Colwell (2000) recently reported that commercial nucleases have no virucidal properties (i.e., do not cause virus lysis). Moreover, Danovaro et al. (2001) demonstrated that virus counts from marine sediments were increased by the use of nuclease treatment. Therefore, the likelihood of viral DNA contamination is low.

The nuclease method for extracellular DNA extraction yielded significantly higher extracellular DNA concentrations than the procedure of Ogram (1987) in both muddy and sandy sediments. Therefore, the nuclease treatment can be considered the most effective available method for extracellular DNA extraction from marine sediments; at the same time, this procedure could be used to minimize extracellular DNA contamination.

Ecological significance and fate of extracellular DNA in sediments: HDNA concentrations reported in this study are high, confirming previous findings of the presence of large amounts of DNA in surface marine sediments. Dell'Anno et al. (1998) and Danovaro et al. (1999) reported that a significant fraction of sedimentary DNA is extracellular (i.e., >80% not associated with living biomass). Because the nonbound (soluble) fraction of DNA accounted only for <5% of the total HDNA concentration, it can be concluded that >95% of extracellular DNA concentration was bound to the sediment matrix.

The occurrence of extracellular DNA in marine sediments is the result of in situ DNA production by cell death and lysis and external DNA inputs from the photic layer (Bailiff and Karl 1991; Danovaro et al. 1999; Dell'Anno et al. 1999). Once DNA enters the sediments, it is adsorbed onto inorganic constituents of sediments, such as quartz and clay minerals, and organic macromolecules, which might alter its bioavailability and diagenesis (Lorenz and Wackernagel 1994; Nielsen et al. 1998).

The observed accumulation of high extracellular DNA concentrations in marine sediments might have important ecological implications. Some authors have stressed the potential relevance of extracellular DNA as a source of organic N, P, and nucleotides for bacterial growth and turnover (Dell'Anno et al. 1998; Danovaro et al. 1999), whereas others hypothesized that extracellular DNA might also represent

a source of genetic material, participating in gene transfer via natural transformation (Stewart and Sinigalliano 1990). Finally, nucleic acids, being P-rich molecules and accounting for a nonnegligible fraction of the organic phosphorous pool in the sediment (Ingall et al. 1990), might play a role in biogeochemical processes involving P regeneration (Kolowith et al. 2001). In this regard, we found that phosphorous associated with HDNA (on average 1.3 ± 0.3 μg g^{-1}) accounted for ~5% of the total organic phosphorous pool in PAP sediments (on average 32.6 ± 5.2 μg g^{-1}).

The contribution of HDNA concentrations to the total DNA pool strongly decreased with depth in the sediment (from ~70% in the top 5 mm to ~10% in the 100–150-mm sediment horizon). This result suggests that DNA in marine sediments is only partially degradable by microbial nucleases (on average, in the top 15 cm of the sediment, >50% of extracellular DNA was refractory; Table 1). This finding might explain why DNA accumulates in surface sediments and suggests that DNA might influence rates of P recycling in marine ecosystems.

Moreover, extracellular DNA binding onto the sediment matrix protects DNA from nuclease degradation but does not prevent transformation (Khanna and Stotzky 1992; Demaneche et al. 2001). Therefore, bound but nondigestible extracellular DNA might represent a reservoir of genetic information (Demaneche et al. 2001).

Data presented here provide the first information on the fate and base composition of extracellular DNA in marine sediments. We found that deoxycytidine and deoxyguanosine dominated and that all bases displayed evidence of temporal changes and spatial trends. In the top 5 mm of the sediment collected in September 1996, both dG : dC and dA : dT molar ratios were close to 1 (1.1 and 1.3, respectively) as expected for double helix DNA (i.e., native DNA). Conversely, in March 1998, both dG : dC and dA : dT ratios increased (to 3 and 1.6, respectively), suggesting the presence of a more heterogeneous DNA pool (i.e., native and single helix DNA). Such differences could be related to the temporal changes of DNA fluxes observed in this area by Dell'Anno et al. (1999): molar ratios of dG : dC and dA : dT close to 1 were coupled to large inputs of primary organic matter from the photic layer (Fabiano et al. 2001). Because standards of deoxynucleosides added to sediments were not selectively adsorbed, changes in base composition are likely to be dependent on selective degradation or utilization of extracellular DNA or both.

HDNA concentrations decreased significantly with depth in the sediment core and displayed a clear change in base composition. In particular, deoxycytidine content increased with increasing depth in the sediment, whereas deoxyadenosine decreased. Such a pattern could reflect changes in G+C content of the ambient microbial community (which is one of the sources of extracellular DNA, Jimenez 1990) but could be also the result of a selective degradation of extracellular DNA bases. In this regard, it has been demonstrated that bacterial degradation of adenosine monophosphate in marine sediments is much faster than cytidine monophosphate (Therkildsen at al. 1996). Moreover, recent studies reported that extracellular nuclease produced by the fungus *Basidiobolus haptosporus* hydrolyzed DNA with preferential liberation of 5′-deoxyguanylate monophosphate, whereas DNA containing cytosine linkages were refractory to nuclease cleavage (Desai and Shankar 2000). In light of these findings, our results suggest that extracellular DNA in marine sediments might be selectively degraded, utilized, or both.

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References

ATLAS, R. M. 1993. Extraction of DNA from soils and sediments, p. 261–266. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], Handbook of methods in aquatic microbial ecology. Lewis Publishers.

BAILIFF, D. M., AND D. M. KARL. 1991. Dissolved and particulate DNA dynamics during a spring bloom in the Antarctic Peninsula region, 1986–87. Deep-Sea Res. I **38:** 1077–1095.

DANOVARO, R., A. DELL'ANNO, A. PUSCEDDU, AND M. FABIANO. 1999. Nucleic acid concentrations (DNA, RNA) in the continental and deep-sea sediments of the eastern Mediterranean: Relationships with seasonal varying organic inputs and bacterial dynamics. Deep-Sea Res. I **46:** 1077–1094.

———, ———, A. TRUCCO, M. SERRESI, AND S. VANUCCI. 2001. Determination of virus abundance in marine sediments. Appl. Environ. Microbiol. **67:** 1384–1387.

DAVISON, J. 1999. Genetic exchange between bacteria in the environment. Plasmid **42:** 73–91.

DELL'ANNO, A., M. FABIANO, G. C. A. DUINEVELD, A. KOK, AND R. DANOVARO. 1998. Nucleic acid (DNA, RNA) quantification and RNA/DNA ratio determination in marine sediments: Comparison of spectrophotometric, fluorometric and high perfor-

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mance liquid chromatography methods and estimation of detrital DNA. Appl. Environ. Microbiol. **64:** 3238–3245.

———, ———, M. L. MEI, AND R. DANOVARO. 1999. Pelagic–benthic coupling of nucleic acids in an abyssal location of the northeastern Atlantic Ocean. Appl. Environ. Microbiol. **65:** 4451–4457.

DEMANECHE, S., L. JOCTEUR-MONROZIER, H. QUIQUAMPOIX, AND P. SIMONET. 2001. Evaluation of biological and physical protection against nuclease degradation of clay-bound plasmid DNA. Appl. Environ. Microbiol. **67:** 293–299.

DESAI, N. A., AND V. SHANKAR. 2000. Purification and characterization of the single-strand-specific and guanylic-acid-preferential deoxyribonuclease activity of the extracellular nuclease from *Basidiobolus haptosporus*. Eur. J. Biochem. **267:** 5123–5135.

FABIANO, M., A. PUSCEDDU, A. DELL'ANNO, M. ARMENI, S. VANUCCI, R. LAMPITT, G. A. WOLFF, AND R. DANOVARO. 2001. Fluxes of phytopigments and labile organic matter to the deep ocean in the NE Atlantic Ocean. Prog. Oceanogr. **50:** 89–104.

FROSTEGARD, A., AND OTHERS. 1999. Quantification of bias related to the extraction of DNA directly from soils. Appl. Environ. Microbiol. **65:** 5409–5420.

GARKO, K. A., AND G. J. STEWART. 1994. The effect of divalent cations on the binding of DNA to marine sediment. Microb. Release **2:** 191–199.

HEAD, I. M., J. R. SAUNDERS, AND R. W. PICKUP. 1998. Microbial evolution, diversity and ecology: A decade of RNA analysis of uncultivated microorganisms. Microb. Ecol. **35:** 1–21.

HURT, R. A., X. QIU, L. WU, Y. ROH, A. V. PALUMBO, J. M. TIEDJE, AND J. ZHOU. 2001. Simultaneous recovery of RNA and DNA from soils and sediments. Appl. Environ. Microbiol. **67:** 4495–4503.

INGALL, E. D., P. A. SCHROEDER, AND R. A. BERNER. 1990. The nature of organic phosphorous in marine sediments: New insights from ^{31}P NMR. Geochim. Cosmochim. Acta **54:** 2617–2620.

JIMENEZ, L. 1990. Molecular analysis of deep-subsurface bacteria. Appl. Environ. Microbiol. **56:** 2108–2113

JØRGENSEN, N. O. G., AND C. S. JACOBSEN. 1996. Bacterial uptake and utilization of dissolved DNA. Aquat. Microb. Ecol. **11:** 263–270.

JUNIPER, S. K., M. A. CAMBON, F. LESONGEUR, AND G. BARBIER. 2001. Extraction and purification of DNA from organic rich subsurface sediments. Mar. Geol. **174:** 241–247.

KARL, D. M., AND M. D. BAILIFF. 1989. The measurement and distribution of dissolved nucleic acids in aquatic environments. Limnol. Oceanogr. **34:** 543–558.

KHANNA, M., AND G. STOTZKY. 1992. Transformation of *Bacillus subtilis* by DNA bound on montmorillonite and effect of DNase on the transforming ability of bound DNA. Appl. Environ. Microbiol. **58:** 1930–1939.

KOLOWITH, L. C., E. D. INGALL, AND R. BENNER. 2001. Composition and cycling of marine organic phosphorous. Limnol. Oceanogr. **46:** 309–320.

KROER, N., N. O. G. JORGENSEN, AND R. B. COFFIN. 1994. Utilisation of dissolved nitrogen by heterotrophic bacterioplankton: A comparison of three ecosystems. Appl. Environ. Microbiol. **60:** 4116–4123.

LORENZ, M. G., AND W. WACKERNAGEL. 1987. Adsorption of DNA to sand and variable degradation rates of adsorbed DNA. Appl. Environ. Microbiol. **53:** 2948–2952.

———, AND ———. 1994. Bacterial gene transfer by natural genetic transformation in the environment. Microbiol. Rev. **58:** 563–602.

MILLER, D. N., J. E. BRYANT, E. L. MADSEN, AND W. C. GHIORSE. 1999. Evaluation and optimization of DNA extraction and purification for soil and sediment samples. Appl. Environ. Microbiol. **65:** 4715–4724.

NIELSEN, K. M., A. M. BONES, K. SMALLA, AND J. D. VAN ELSAS. 1998. Horizontal gene transfer from transgenic plants to terrestrial bacteria—a rare event. FEMS Microbiol. Rev. **22:** 79–103.

NOVITSKY, J. A., AND D. M. KARL. 1985. Influence of deep ocean sewage outfalls on the microbial activity of the surrounding sediments. Appl. Environ. Microbiol. **50:** 1464–1473.

OGRAM, A., G. S. SAYLER, D. GUSTIN, AND R. J. LEWIS. 1987. The extraction and purification of microbial DNA from sediments. J. Microbiol. Methods **7:** 57–66.

PAUL, J. H., M. F. DEFLAUN, AND W. H. JEFFREY. 1988. Mechanisms of DNA utilization by estuarine microbial populations. Appl. Environ. Microbiol. **54:** 1682–1688.

———, W. H. JEFFREY, A. W. DAVID, M. F. DEFLAUN, AND L. H. CAZARES. 1989. Turnover of extracellular DNA in eutrophic and oligotrophic freshwater environments of Southwest Florida. Appl. Environ. Microbiol. **55:** 1823–1828.

PURDY, K. J., T. M. EMBLEY, S. TAKII, AND D. B. NEDWELL. 1996. Rapid extraction of DNA and rRNA from sediments by a novel hydroxyapatite spin-column method. Appl. Environ. Microbiol. **62:** 3905–3907.

ROMANOWSKI, G., M. G. LORENZ, AND W. WACKERNAGEL. 1991. Adsorption of plasmid DNA to mineral surfaces and protection against DNase I. Appl. Environ. Microbiol. **57:** 1057–1061.

STEWART, G. J., AND C. D. SINIGALLIANO. 1990. Detection of horizontal gene transfer by natural transformation in native and introduced species of bacteria in marine and synthetic sediments. Appl. Environ. Microbiol. **56:** 1818–1824.

TAMAOKA, J., AND K. KOMAGATA 1984. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microbiol. Lett. **25:** 125–128.

THERKILDSEN, M. S., G. M. KING, AND B. A. LOMSTEIN. 1996. Urea production and turnover following the addition of AMP, CMP, RNA and a protein mixture to a marine sediment. Aquat. Microb. Ecol. **10:** 173–179.

TREVORS, J. T. (1996) Nucleic acids in the environment. Curr. Opin. Biotechnol. **7:** 331–336.

TURK, V., A.-S. REHNSTAM, E. LUNDBERG, AND A. HAGSTROM. 1992. Release of bacterial DNA by marine nanoflagellates, an intermediate step in phosphorus regeneration. Appl. Environ. Microbiol. **58:** 3744–3750.

WOMMACK, K. E., AND R. R. COLWELL. 2000. Virioplankton: Viruses in aquatic ecosystem. Microbiol. Mol. Biol. Rev. **64:** 69–114.

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