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Measurement of Bacterial Growth Rates in Subsurface Sediments Using the Incorporation of Tritiated Thymidine into DNA

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Abstract. Microbial growth rates in subsurface sediment from three sites were measured using incorporation of tritiated thymidine into DNA. Sampling sites included Lula, Oklahoma, Traverse City, Michigan, and Summit Lake, Wisconsin. Application of the thymidine method to subsurface sediments required (1) thymidine concentrations greater than 125 nM, (2) incubation periods of less than 4 hours, (3) addition of SDS and EDTA for optimum macromolecular extraction, and (4) DNA purification, in order to accurately measure the rate of thymidine incorporation into DNA. Macromolecule extraction recoveries, as well as the percentage of tritium label incorporated into the DNA fraction, were variable and largely dependent upon sediment composition. In general, sandy sediments yielded higher extraction recoveries and demonstrated a larger percentage of label incorporated into DNA than sediments that contained a high silt-clay component. Reported results also indicate that the acid-base hydrolysis procedure routinely used for macromolecular fractionation in water samples may not be routinely applicable to the modified sediment procedure where addition of SDS and EDTA are required for macromolecule extraction. Growth rates exhibited by subsurface communities are relatively slow, ranging from 5.1 to 10.2 $\times$ 10^5 cells g^{-1} day^{-1}. These rates are 2–1,000-fold lower than growth rates measured in surface sediments. These data lend support to the supposition that subsurface microbial communities are nutritionally stressed.

Introduction

Considerable research has been conducted on the growth and metabolism of microbial life in aquatic and soil environments, but few studies have addressed metabolism and growth in subsurface environments. Studies focusing on population density [11, 39], diversity [4, 12], and community structure [6, 38, 39] have revealed that subsurface environments are inhabited by diverse communities of bacteria. However, only a small percentage of the community appears capable of metabolic activity [11, 39]. Heterotrophic activity [6, 34–36] and biodegradation [16, 35, 36, 40] studies have delineated the role of indigenous communities in degradation of both organic and inorganic pollutants that permeate subsurface sediment.

As it has not been possible to draw specific conclusions regarding the growth of subsurface bacterial communities from existing studies, direct measurement of bacterial growth rates is essential. Subsurface bacterial communities appear to be adapted for survival under oligotrophic conditions which characteristically exist in pristine subsurface sediments. Wilson et al. [40] proposed that bacterial communities subsist in these oligotrophic sediments by metabolizing residual organic matter which percolates down from the surface. Additional indications of the nutritionally stressed status of the microflora are found in studies of the morphology and ultrastructure of individual subsurface bacteria [2] and analysis of cellular components of sediment communities [6, 30, 37, 38]. Estimation of microbial growth rates will not only help describe the physiological state of subsurface microflora, but will also increase our understanding of the functioning of natural microbial communities in aquifer restoration strategies.

The thymidine incorporation method developed by Fuhrman and Azam [8] has been instrumental in estimating bacterial growth rates in natural environments. Secondary production in both marine [8, 9, 23, 27, 41] and freshwaters [7, 15, 17, 23, 25, 26] has been measured. Fewer [^{3}H]-thymidine studies have examined growth and production in marine and freshwater sediments [5, 7, 18–20, 33] and none, to date, have addressed the growth of subsurface sediment bacteria. The tritiated thymidine method involves the short-term measurement of [methyl-^{3}H]-thymidine incorporation into bacterial DNA in order to estimate the rate of DNA synthesis. The central assumption is that the rate of nucleic acid synthesis is directly proportional to cellular growth [21].

The subsurface is quite different from other environments where tritiated thymidine methodology has been employed; these sediments are generally low in total organic carbon and nutrients [31, 32]. We were interested in assessing applicability of the method to subsurface sediments. This was accomplished in terms of evaluating existing modifications of the [^{3}H]-thymidine approach, specifically in terms of quantitative extraction of DNA, and subsequently applying an appropriate method to subsurface sediment for estimation of bacterial growth rates.

Materials and Methods

Chemicals

Thymidine and DNA were obtained from Sigma Chemical Co., St. Louis, Missouri; [methyl-^{3}H]-thymidine (>62 Ci mmol^{-1}) was obtained from ICN Pharmaceuticals Inc., Irvine, California. All other chemicals used were of reagent grade or better and were obtained from Fisher Scientific Co., Pittsburgh, Pennsylvania. Norganic cartridge-treated Milli-Q water (Millipore Corp., Bedford, Massachusetts) was used throughout this study.

Description and Collection of Subsurface Samples

Subsurface sediments were obtained from three locations: Lula, Oklahoma; Traverse City, Michigan; and Summit Lake, Wisconsin. The procedures for collection and aseptic handling are described

by Wilson et al. [40]. The Lula samples were previously characterized as 0–65% sand, 19–53% silt, and 16–35% clay [29]. The composition of sediments used in this study are listed in Table 3.

The Traverse City sites, 44G and 44E, were characterized as primarily sand (>95%) with some clay-silt component by the standard method for particle-size analysis [10]. Identification numbers for both Lula and Traverse City samples are those designated by staff scientists at the U.S. Environmental Protection Agency, Robert S. Kerr Environmental Research Laboratory, Ada, Oklahoma.

Summit Lake, Wisconsin samples were characterized by the standard method for particle-size analysis [10]. Descriptions of samples used in this study include North well (5.4 m)—sand 90.86%, silt 8.49%, and clay 0.65%; North well (17 m)—sand 91.49%, silt 8.19%, and clay 0.32%; South well (7.8 m)—sand 86.53%, silt 9.46%, and clay 3.98%; South well (18 m)—sand 96.18%, silt 2.77%, and clay 1.05%.

Incorporation of [^{3}H]-Thymidine: Extraction Methods

The efficacy of two previously described methods used in estimating bacterial growth rates in marine and freshwater sediments was evaluated in terms of DNA extraction efficiency (Fig. 1).

Method 1 is similar to that described by Moriarty and Pollard [19] as applied to seagrass sediment. One to three grams (wet weight) of sediment, prepared as a slurry (2 ml water, 3 g sediment) was added to five replicate centrifuge tubes and incubated with 125 nM [methyl-^{3}H]-thymidine at 16°C in the dark. Controls for each sediment sample were prepared by autoclaving sediment and adding NaOH to a final concentration of 0.6 N prior to incubation with tritiated thymidine. Samples treated in this manner gave control counts of less than 500 cpm per filter. Following a 3 hour incubation period with [^{3}H]-thymidine, samples were treated with NaOH to a final concentration of 0.6 N to terminate uptake of label by cells. DNA was extracted and RNA hydrolyzed for 18 hours at 37°C. Following extraction and hydrolysis, the slurry was centrifuged at 5,900 × *g* for 10 min to settle suspended material. The supernatant was removed, neutralized with HCl, and trichloroacetic acid (TCA) was added to a final concentration of 5%. The solution was then chilled on ice for 45 min. TCA insoluble material was collected on Type HA nitrocellulose Millipore filters (0.45 μm pore size). An additional 5 ml of ice-cold 5% TCA was used to rinse the test tube originally containing the supernatant. The filters were then rinsed with 20 ml of 5% TCA and removed to scintillation vials. To prepare for scintillation counting, 0.3 ml of 1.0 N HCl was added to the vials and filters, which were then placed in a boiling water bath for 30 min to hydrolyze DNA [8]. After cooling, scintillation fluor containing methyl cellosolve was added, and tritium incorporation was determined using scintillation spectrometry. Counts were corrected for quenching with an external standard. Control sample counts were subtracted from experimental counts to correct for adsorption to sediment particles.

A modification of the above procedure, method 2, was also evaluated for DNA extraction efficiency. This method is similar to that described by Findlay et al. [7] with modifications to optimize recovery of DNA. Following slurry preparation and incubation with [^{3}H]-thymidine, incubation was terminated by the addition of NaOH to a final concentration of 0.3 N. Final concentrations of 25 mM EDTA and 0.1% sodium dodecyl sulfate (SDS) were added as a means of increasing the extraction recovery of DNA [7]. DNA was extracted and RNA was hydrolyzed for 12 hours at 25°C. The slurry was then centrifuged at 5,900 × *g* for 10 min to settle suspended material and supernatants removed to test tubes on ice. Pellets were washed with extraction reagents, and supernatants were again removed to test tubes on ice. Combined supernatants were neutralized with HCl, and final concentrations of aqueous DNA and thymidine (4 and 17 μg ml^{-1}) were added. The solutions were acidified to 5% with TCA and chilled 45 min on ice. The precipitate was collected on Millipore HA filters and treated as described above.

The relative efficiency of DNA extraction from sediments was estimated by comparing the recovery of radioactivity in TCA precipitable material from prelabeled bacterial cells using the protocol of Fallon et al. [5]. A pond water suspension, enriched with glucose and yeast extract, was aerated until growth at 25°C was visible. Tritiated thymidine was added to 15 nM and cells were allowed to incorporate tritium label for 3–4 hours. Cells were recovered by centrifugation

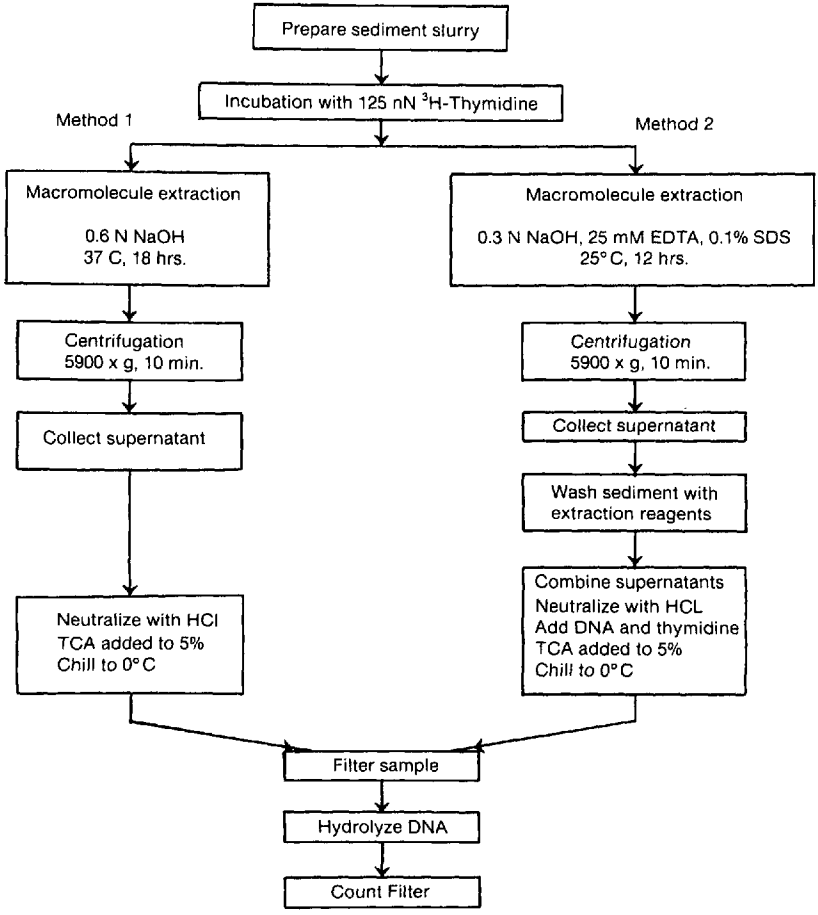

Fig. 1. Schematic of extraction methods.

and pellets were washed repeatedly with 0.1 M standard saline citrate, pH 7.2 (SSC). To test extraction efficiency, cells were resuspended in SSC and added to sediment or pond water. Samples were thoroughly mixed and extracted within 2 hours after the procedures of Fuhrman and Azam [8] for aqueous suspensions, or by method 1 and 2 for sediment samples. Recovery by the Fuhrman and Azam procedure was arbitrarily assigned a value of 100%.

Macromolecular Fractionation

In order to examine the possibility that some tritiated thymidine was being incorporated into macromolecular fractions other than DNA, an acid-base hydrolysis procedure [25, 27] for separation of DNA, RNA, and protein was performed using supernatants of sediment slurries obtained by centrifugation.

Following a 3 hour incubation with [^{3}H]-thymidine (125 nM), method 2 was employed for DNA extraction and RNA hydrolysis. Supernatants were collected following centrifugation, and acid-base hydrolysis was performed as follows: One set of samples (i) was extracted in an equal volume

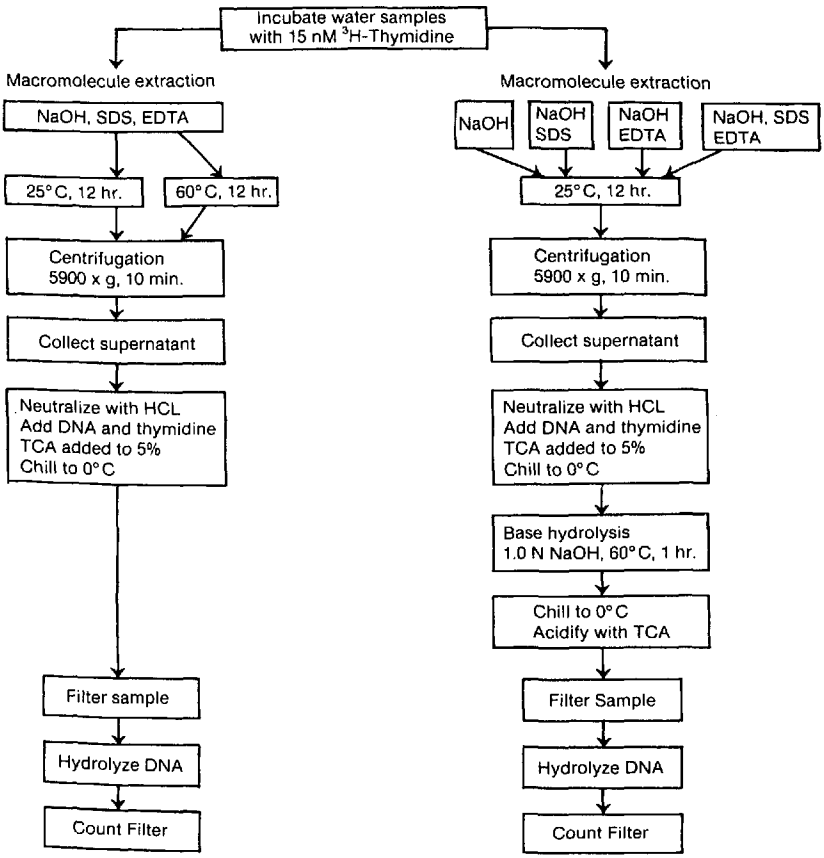

Fig. 2. Schematic for determination of the effect of SDS and EDTA on the base-hydrolysis scheme when used with modified sediment technique (method 2).

of 10% ice-cold TCA; a second set (ii) was extracted with 1.0 N NaOH (final concentration) at 60°C for 1 hour, chilled, and re-acidified to 5% with TCA; and a third set (iii) of samples was acidified to 20% with TCA and heated at 95–100°C for 30 min. All samples were chilled prior to filtration and treated as previously described for determination of radioactivity. Labeled DNA was then calculated by subtraction as described by Riemann [25]. Fractions contain (i) DNA-RNA-protein, (ii) DNA-protein, and (iii) protein, respectively. The relative labeling of macromolecules is then presented as a percentage of the label appearing in the initial (i) cold-TCA precipitate.

Effect of SDS and EDTA on the Acid-Base Hydrolysis Scheme

In order to evaluate applicability of the acid-base hydrolysis scheme for determining tritium labeling of the DNA fraction, when used in conjunction with method 2, the following two experiments were conducted.

First, pond water samples rather than sediment were incubated with 15 nM [^{3}H]-thymidine. Using method 2, macromolecules were extracted from two sets of samples at 25 and 60°C for 12 hours to determine the effect of elevated extraction temperature on dpm recovery in total TCA precipitable material (Fig. 2). Concomitantly, macromolecules in four sets of samples were extracted

in both the presence and absence of 0.1% SDS and 25 mM EDTA. Extraction was performed at 25° for 12 hours and the effect of extraction reagents (SDS and EDTA) on dpm recovery following base-hydrolysis was assessed.

Second, pond water samples were incubated for 1 hour with 0.26 μCi [U-^{14}C]-glutamate (New England Nuclear, Boston, Massachusetts) to label the protein fraction specifically. Each of three sets of water samples was then acidified to 5% with TCA and chilled. One set of samples was filtered and counted to represent total radioactivity incorporated into macromolecules. Protein was extracted from the other two sets of samples (using the acid-hydrolysis scheme) in the absence and presence of 25 mM EDTA and 0.1% SDS. Samples were then chilled prior to filtration. Of the total glutamate label incorporated into macromolecules, 60% was determined to be present in the protein fraction, as determined by acid-hydrolysis in the absence of SDS and EDTA. The effect of SDS and EDTA on the acid-hydrolysis scheme was determined by comparison of dpm recoveries in the absence and presence of the reagents.

Time Course of [^{3}H]-Thymidine Incorporation into DNA

A time course of tritiated thymidine incorporation into DNA was determined by incubating sediments having different silt-clay composition with 125 nM tritiated thymidine for periods of 3–360 min. DNA was extracted, RNA was hydrolyzed, and supernatants were treated as previously described by method 2.

Enumeration of Subsurface Microorganisms

Bacterial abundance was determined by epifluorescence microscopy of acridine orange-stained cells as described by Hobbie et al. [13] or Ghiorse and Balkwill [11]. Samples were preserved in 0.1–0.5% formaldehyde and refrigerated until counted.

Results and Discussion

Concentration Dependence

Previous investigators have determined the optimum [^{3}H]-thymidine concentration as that at which further additions result in no further increased radioactivity in DNA [8, 26]. We have found that additions of [^{3}H]-thymidine up to 100 nM yielded increases in the amount of tritium incorporated. When concentrations greater than 100 nM were employed, a constant incorporation rate was noted for all samples tested. In sediment samples, much of the added thymidine is probably absorbed to sediment components, with the actual radiolabeled substrate concentration available for bacterial incorporation being quite small [22]. It is necessary to ensure that the total concentration of radiolabeled thymidine in the incubation is present at saturating concentrations for two reasons. First, to overcome isotopic dilution due to the presence of extracellular dissolved unlabeled thymidine; and second, to prevent intracellular isotopic dilution by inhibiting de novo synthesis of deoxythymidine [20]. Moriarty and Pollard [21, 24] proposed that during slow growth, which is demonstrated by subsurface communities in this study, the radiolabeled thymidine supplied should be sufficient to supply all the requirements for DNA synthesis, thus feedback inhibition of de novo synthesis will be inhibited.

Table 1. Comparison of extraction recoveries

Sample	% silt-clay	Recovery					
		Method[a]		Method 1[b]		Method 2[c]	
		dpm	% Eff.	dpm	% Eff.	dpm	% Eff.
Pond water	0	209,412	100	—	—	—	—
9HH3	53	—	—	419	0.2	127,532	60.9
9JJ1	57	—	—	ND[d]	ND	141,981	67.8
9HH9	78	—	—	ND	ND	105,344	50.3
9JJ3	82	—	—	ND	ND	150,148	71.7
44G	<5	—	—	36,857	17.6	156,221	74.6
44E	<5	—	—	37,694	18.0	151,405	79.8

[a] Fuhrman and Azam [8]
[b] DNA extracted and RNA hydrolyzed for 18 hours with 0.6 N NaOH at 37°C
[c] DNA extracted and RNA hydrolyzed for 12 hours with 0.3 N NaOH, 0.1% SDS and 25 mM EDTA at 25°C. Carrier DNA and unlabeled thymidine were added to increase precipitation of macromolecules
[d] Not determined

Extraction Efficiency

Data showing the extraction recoveries for TCA-precipitable macromolecules from subsurface sediments are shown in Table 1. Following addition of prelabeled cells (249,300 dpm) to replicate pond water samples, approximately 84% of the added counts (209,412 dpm) were recovered as TCA-precipitable macromolecules using the method of Fuhrman and Azam [8]. Relative to the recovery of radioactivity using the protocol of Fuhrman and Azam, which represents the total macromolecule recovery in the absence of interfering sediment, the 0.6 N NaOH extraction of sediments (method 1) recovered less than 1 and 18% from silt-clay and sandy sediments, respectively. Method 2 showed increased recoveries averaging 62 and 77% from silt-clay and sandy sediments, respectively. Because both method 1 and 2 hydrolyse RNA during macromolecule extraction with NaOH, and the method of Fuhrman and Azam represents total macromolecule recovery (RNA hydrolysis does not occur), relative extraction efficiencies are conservative estimates. The increased recovery by method 2 is consistent with results of Findlay et al. [7], who found that recovery of TCA precipitable material increased to 74% using the milder extraction protocol (0.3 N NaOH; 0.1% SDS; 25 mM EDTA) with sandy, high organic sediment. Contrary to the findings of Findlay et al., we found that increasing extraction time to 18 hours was not deleterious to macromolecule recovery in subsurface sediments (data not shown). Interestingly, we found that extraction efficiencies differed markedly between sediment types, with silt-clay sediments (>30% silt-clay) generally yielding lower extraction recoveries than sandy sediments. This finding is contrary to that of Fallon et al. [5] who noted lower extraction recoveries of radioactivity in extracts from sandy sediments (53%) than from clay-silt sediments (89%). Recent reports have noted montmorillonite clay as the most important factor controlling DNA sorption to soils at pH below 7.0 (A. Ogram, D. Gustin, and G. S. Sayler, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, Q143, pp 307).

Using method 2, we found addition of carrier DNA and unlabeled thymidine necessary for optimal macromolecule recovery. DNA promotes precipitation of low concentrations of radiolabeled nucleic acids in the TCA extract, and thymidine minimizes control values by reducing nonspecific binding of tritiated thymidine. Others have also found that these compounds enhanced macromolecule collection [5, 33].

Macromolecular Fractionation

The specificity of [methyl-^{3}H]-thymidine labeling of macromolecules was examined. Previous experiments with macromolecular fractionation have shown appreciable amounts of tritium incorporation into protein [9, 14], RNA [14, 25, 41], and cellular constituents including lipids [28, 41] as well as DNA. Witzel and Graf [41] demonstrated that 50% of the added label may be introduced into cellular substances other than nucleic acids due to metabolic channeling of the tritium label during cell metabolism.

When applied to water samples, the acid-base hydrolysis scheme accurately accomplishes macromolecular fractionation [25–27]. Specific DNase, RNase, and protease treatments have been used to confirm fraction contents, with the finding that the major portions of RNA, DNA, and protein are found in expected hydrolysis fractions [25]. However, base-hydrolysis does not appear to be appropriate for macromolecular fractionation when used in conjunction with our modified sediment technique, which requires SDS and EDTA addition for optimum macromolecule extraction.

Table 2 shows that the introduction of SDS or EDTA into the base-hydrolysis scheme was responsible for a 41 and 37% reduction, respectively, in dpm recovered in the base-hydrolysis fraction. A 55% reduction in dpm recovery resulted when both reagents were present during base-hydrolysis. Although a 14% reduction in counts recovered in the total TCA precipitable material was due to elevated extraction temperature alone, presumably the base-hydrolysis treatment (previously described as specific for RNA hydrolysis) also hydrolyzes DNA when SDS and EDTA are present.

The presence of SDS and EDTA is not detrimental to the acid-hydrolyzed fraction (previously described as specific for DNA hydrolysis) as demonstrated by the results using ^{14}C-glutamate-labeled water samples. Following acid-hydrolysis in the presence of SDS and EDTA, no reduction in radioactivity associated with protein was observed relative to the protein fraction obtained without SDS and EDTA present. This establishes that acid-hydrolysis remains specific for DNA hydrolysis and does not hydrolyze protein when performed in the presence of SDS and EDTA. Therefore, this fraction remains applicable for determination of macromolecule fractions when method 2 is employed.

Table 3 lists the relative labeling of subsurface bacterial DNA and protein from six sites. Method 2 was used for DNA extraction and RNA hydrolysis. Radioactivity incorporated into the DNA fraction was determined by subtraction of the acid-hydrolysis fraction from the total radioactivity incorporated into cold TCA precipitate. As described above, the base-hydrolysis fraction is not applicable due to nonspecific hydrolysis of DNA as well as RNA in the

Table 2. Effects of temperature, SDS, and EDTA on the base-hydrolysis fraction

Treatment	DPM recovery
Temperature[a]	
25°C	170,000
60°C	147,013
Extraction reagents[b]	
NaOH	91,683
NaOH + SDS	42,486
NaOH + EDTA	45,940
NaOH + SDS + EDTA	28,503

[a] Counts are in total TCA precipitable material
[b] Counts are recovered following base hydrolysis

Table 3. Percentage of macromolecules labeled with tritiated thymidine

Sediment sample	Depth (m)	TOC %	Sediment composition			Macromolecular labeling	
			% Sand	% Clay	% Silt	% DNA	% Protein
9HH3	1.90	0.11	18	35	47	84	16
9JJ1	3.15	0.04	27	30	43	87	13
9HH9	3.80	0.04	44	34	22	66	34
9JJ3	5.00	0.04	70	12	18	65	35
44G	9.90	ND[a]	>95	ND	ND	98	2
44E	7.30	ND	>95	ND	ND	95	5

[a] Not determined

presence of SDS and EDTA, and therefore is not used in this calculation. Additionally, hydrolysis of RNA is accomplished during the 12 hour macromolecule extraction with 0.3 N NaOH [7]; therefore, little or no tritium label should be associated with the RNA fraction.

The variability of the proportion of label into DNA ranged from 65 to 98% in silt-clay and sandy sediments, respectively. The relative distribution of radioactivity in the DNA and protein fractions suggests that tritiated thymidine is preferentially used for DNA synthesis by subsurface bacterial communities. That labeled proteins are present (2–35% of total radioactivity incorporated) indicates that some intracellular degradation of thymidine by thymidine phosphorylase does occur with subsequent degradation of thymine [3]. Radioactivity in the protein fraction does not interfere with the estimation of growth rates using thymidine incorporation. It does, however, confirm the need to measure label in the DNA fraction specifically, rather than in the total cold TCA precipitate. This point has been made previously by other researchers working with different environmental samples [7, 14, 21, 22, 41]. The higher percentage of label incorporated into DNA in sandy sediments may reflect greater access to trace carbon and nutrients by indigenous organisms due to increased permeability of sandy sediment. In silt-clay sediments, sorption processes may reduce nutrient availability to bacterial communities. Thus, these organisms

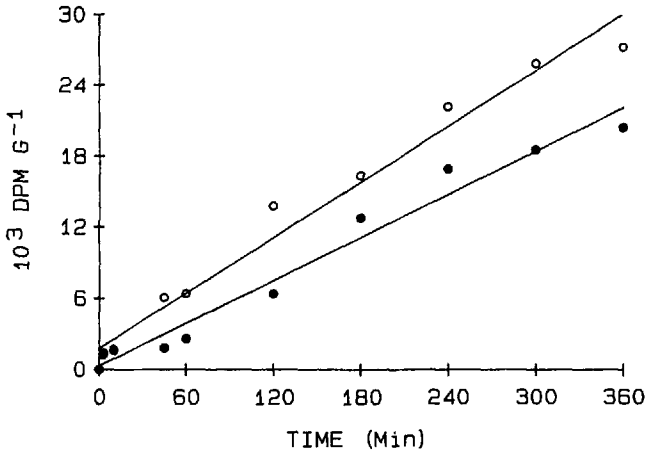

Fig. 3. Time course of incorporation of [methyl-^{3}H]-thymidine into DNA in (●) >30% silt-clay and (O) sandy (<5% silt-clay) subsurface sediment.

are not as apt to be in a constant growth state as those associated with more permeable sandy sediments. Robarts et al. [28] suggested that since the relative labeling of bacterial macromolecules with tritiated thymidine varies between environments depending upon growth and nutrient conditions, it may be useful to use a fractionation scheme to examine the metabolic state of heterogenous bacterial communities from different environmental matrices.

Time Course

The time course of [^{3}H]-thymidine incorporation into DNA was linear for 2–3 hours for both sandy and silt-clay sediments (Fig. 3). This indicates that radiolabeled thymidine was not depleted during the linear time period and that incorporation rates were constant at 16°C. These results are consistent with those of Tobin and Anthony [33] who observed linear incorporation of tritiated thymidine in lake sediments for up to 3 hours at 4°C. Others have observed a departure from linearity within 30 min in seagrass sediment [19]. However, the sediment employed was affected by input of photosynthetically derived DOC [20], which indicates the system was eutrophic rather than oligotrophic, and growth rates were the highest for a particulate system (Table 5). In contrast, subsurface sediment bacteria having little organic carbon present for utilization, are not likely to have substantially varying growth rates on the short time scale used during our incubations.

Growth Rate Estimates

Growth rates were calculated by using a conversion factor of 2.0×10^{18} cells mole^{-1} of [^{3}H]-thymidine incorporated. This conversion factor was developed

Table 4. Bacterial growth rates and abundance in subsurface sediment

Soil sample	Growth rate determined by ^{3}H-Thymidine cells g^{-1} day^{-1} ($\times 10^6 \pm$ SD)	Abundance determined by AODC [11] cells g^{-1} ($\times 10^6 \pm$ SD)
Summit Lake, WI		
North Well		
5.4 m	1.1 ± 2.6	16.2 ± 4.8
17.0 m	0.7 ± 0.6	4.1 ± 2.7 [13]
South Well		
7.8 m	0.8 ± 0.3	17.9 ± 4.2
18.0 m	1.2 ± 0.5	6.1 ± 2.9 [13]
Traverse City, MI		
44G	0.53 ± 0.23	95.8 ± 39.9 [13]
44E	0.51 ± 0.19	10.0 ± 6.7 [13]
Lula, OK		
9HH3	1.02 ± 0.34	7.6 ± 2.2
9HH9	0.71 ± 0.35	3.8 ± 1.2
9JJ1	0.68 ± 0.32	2.8 ± 1.9
9JJ3	0.84 ± 0.54	0.8 ± 0.9

Table 5. Comparison of bacterial growth rates in sediments as measured by tritiated thymidine incorporation

Sample	Bacterial growth rate (cells g^{-1} day^{-1})
Seagrass sediment (surface zone)	7.92×10^7–8.90×10^9 [19]
Marine sediment (near shore)	3.45×10^8–2.76×10^9 [5]
Lake sediment	1.39×10^6–5.94×10^6 [33]
Seagrass sediment	4.80×10^7–6.24×10^8 [20]
Freshwater sediment	3.31×10^6 [7]
Subsurface sediment	5.10×10^5–1.02×10^6

for marine systems by Riemann et al. [26] from a consideration of several parameters including isotopic dilution of radiolabeled thymidine by *de novo* synthesis, thymidine content of bacterial DNA, and the amount of DNA per bacterial cell. The question of variability in conversion factors between different environments remains. It would be of interest to determine this factor within a given subsurface site.

Subsurface bacterial growth rates measured (Table 4) were substantially lower than those reported in other particle-dominated (sediment) environments (Table 5). This is not surprising when one considers the greater input of nutrients in those systems. The rates were also significantly lower than those measured in surface soils (< 1.0 m depth) from the Wisconsin site which are on the average of 1.02×10^8 cells g^{-1} day^{-1}. The reason for the lower activity may be due in

part to the overall lower biomass in subsurface sediments (Table 4). We found no correlation between bacterial numbers and growth rate measurements. This is not surprising as total microscopic counts do not distinguish between metabolically active or inactive cells. The slow growth rates are, no doubt, a consequence of the oligotrophic nature of the subsurface. Similar to seawater microflora, the bacteria may be in a state of reversible dormancy [1] because of nutrient stress. It may be pertinent to examine how manipulation of nutrient levels affects microbial growth in these sediments.

Conclusions

Based on the results of our studies, the following conclusions can be drawn: (1) DNA can be quantitatively extracted from subsurface sediments. (2) It is necessary to routinely purify and measure radioactivity in the DNA fraction due to variability of macromolecule labeling and extaction recoveries with different sediment types. (3) With minor modifications, the thymidine uptake approach can be applied to the subsurface for estimation of microbial community growth rates. (4) Growth of bacteria in subsurface soils is comparatively slow, which may reflect the oligotrophic nature of this environment.

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