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Gene Expression per Gene Dose, a Specific Measure of Gene Expression in Aquatic Microorganisms

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A method for the measurement of specific levels of gene expression that combines target mRNA and target DNA quantitation has been developed. The use of target gene dose as a normalizing factor for mRNA provides an alternative to 16S or 23S rRNA, which are unsuitable for use in the environment because of their presence in nontarget organisms. Both target mRNA and DNA are recovered from replicate samples and detected by using antisense and sense single-stranded RNA gene probes. For efficient mRNA recovery, the use of Millipore Durapore filters and multiple extractions was necessary. Quantitation was performed by radiometric detection by using a β-scanner and comparison of the sample signal against target mRNA and DNA standard curves. This method enabled the measurement of expression of the catechol-2,3-dioxygenase gene (*xylE*) contained on the thermoregulated plasmid pLV1013 in a marine *Vibrio* strain in culture and in the environment. In studies of the relationship between mRNA levels and enzyme activities, the appearance of enzyme activity lagged behind *xylE* mRNA synthesis by an hour after temperature induction. This suggests that mRNA analysis is well suited for determining rapid regulation of microbial gene expression at the transcriptional level in water column microbial populations.

There is a growing interest in detection of microbial gene expression in the environment as a method to monitor microbial activity. The need for measurement of gene expression in the environment has, in part, been created by the use of microorganisms for remediation of contaminated environments and the necessity to monitor their capability to function in the environment (1, 12, 14). Similarly, understanding the regulatory mechanisms controlling microbial activity enables determination of the potential roles microorganisms play in biogeochemical cycling. Traditionally, microbial gene expression has been monitored through the use of specific enzyme assays. Consistent with the efficiency and economy of bacterial growth, control of activity of bacterial genes is primarily exerted on the process of transcription, the synthesis of mRNA (22, 24). However, the cellular content of some enzymes is regulated through translation (24).

Recently, several techniques have been developed for extraction and detection of specific mRNAs from microorganisms in the environment (21, 25, 26). Changes in relative abundances of specific transcripts (mRNA) can be caused by a variety of mechanisms, some of which are (i) increased transcription of the gene, (ii) increase in transcriptional units (gene dosage), (iii) changes in mRNA stability and turnover, and (iv) changes in RNA polymerase pools. Several investigators have monitored changes in gene copy as a response to heavy metal and aromatic hydrocarbon stress (2, 18). These investigations did not attempt to account for increases in activity or mRNA levels caused by changes in gene dose. However, Philippidis et al. (20) found that a 47-fold amplification of plasmid-encoded mercury resistance genes resulted in parallel increases in *mer* mRNAs, *mer* gene products, and Hg^{2+} reduction activity. Gene dose effects have also been documented as the cause for increases in antibiotic resistances in several microorganisms (10, 16, 17).

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In this paper we present a method that will enable measurement of levels of specific mRNAs and account for changes in activity caused by increased transcription or changes in relative gene abundance. This method may be used to study the expression of conserved genes in both indigenous and introduced aquatic microorganisms.

MATERIALS AND METHODS

Bacterial strains and plasmids. The strains and plasmids used in this study are listed in Table 1. Our model for an exogenous gene not likely found in indigenous marine bacteria was the catechol dioxygenase gene (*xylE*), which encodes the enzyme catechol-2,3-dioxygenase (C2,3O). This *xylE* gene was on the broad-host-range plasmid pLV1013 (27), a thermoregulated expression system. This plasmid was naturally transformed into and maintained in *Vibrio* sp. strain WJT-1C (5a). For our model gene encoded by indigenous microorganisms, we chose the large-subunit gene (*rbcL*) for the carboxylating enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCase) (21).

Culture conditions. WJT-1C(pLV1013) was grown on ASWJP plus PY (19) containing 500 μg of kanamycin per ml and 1 mg of streptomycin per ml at 27 to 40°C. *Escherichia coli* B was grown at 37°C in M9 minimal medium (23). All cultures were shaken at 200 rpm. *Synechococcus* sp. strain PCC 6301 was grown in BG-11 medium (5) at 30 to 60 microeinsteins m^{-2} s^{-1} under cool white fluorescent light (Sylvania-GTE) at 22 to 25°C.

Gene expression in seawater. For detection of gene expression in *Vibrio* sp. strain WJT-1C(pLV1013) in seawater, unfiltered seawater was collected from Bayboro Harbor, St. Petersburg, Fla. Mid-log-phase WJT-1C(pLV1013) grown at either 28°C (A_{600} = 0.635) or 40°C (A_{600} = 0.584) was filtered directly or added to 100 ml of seawater and then filtered. A 1.0-ml sample was collected for determination of C2,3O activity, and then diethylpyrocarbonate (DEPC; Sigma Chemical Co., St. Louis, Mo.) was added to 0.1% and a

TABLE 1. Strains and plasmids used in this study.

Strain or plasmid	Characteristic(s)	Source
Strains		
Vibrio sp. strain WJT-1C(pLV1013)	*xylE*	M. Frischer, University of South Florida, St. Petersburg
E. coli B ATCC 11303-B2		J. Rose University of South Florida, Tampa
Synechococcus sp. strain PCC 6301 (ATCC 27144)	*rbcL rbcS*	American Type Culture Collection, Rockville, Md.
Plasmids		
pLV1013	IncQ Sm^r Km^r p_R *xylE* cI857	27
pEPA53	*xylE*	S. Cuskey, Environmental Protection Agency, Gulf Breeze, Fla.
pXYL1	Cb^r *xylE*; pGEM3Z containing *xylE* from pEPA53.	This study
pLC1	Cb^r *rbcL*; pGEM3Z derivative	21

second sample (2.0 ml) was collected for RNA and DNA determinations.

RNA extraction. All reagents were made in either sterile, disposable labware or glassware that had been combusted at 450°C for 4 h. All solutions were made with water that had been treated with DEPC (23). RNA extractions were performed as described by Pichard and Paul (21) with the following modifications. Samples to be extracted were filtered either onto Whatman GF/F filters or onto 0.2-μm-pore-size Millipore Durapore filters, and the filters were placed in sterile 2.2-ml bead beater microcentrifuge tubes (Biospec Products, Bartlesville, Okla.) containing 0.5 ml of GIPS extraction reagent (4M guanidinium isothiocyanate [International Biotechnologies, Inc., New Haven, Conn.], 0.5% sarcosyl, 25 mM sodium citrate [pH 7.0], 0.1 M 2-mercaptoethanol). To each tube, 0.5 ml of water-saturated phenol, 50 μl of 2 M sodium acetate (pH 4.0), 100 μl of chloroform-isoamyl alcohol (49:1), and 0.5 g of glass beads (diameter, 0.1 to 0.15 mm; Biospec Products) were added. The contents of the tubes were beaten for 2 min with a Mini-beadbeater (Biospec Products), cooled on ice for 15 min, and then microcentrifuged for 5 min. The aqueous layer was removed, and the filters were reextracted two more times as described above, using 0.5 ml of GIPS reagent, 50 μl of sodium acetate (pH 4.0), and 100 μl of chloroform-isoamyl alcohol (49:1). The aqueous extracts were then combined, and the RNA was precipitated with 1 volume of isopropanol for 2 h at −20°C. The RNA was pelleted in a microcentrifuge for 10 min and dissolved in DEPC-treated 1 mM EDTA (pH 7.0). The sample was then reprecipitated overnight at −20°C by the addition of 0.1 volume of 2 M sodium chloride, 1 μl of glycogen (20 mg/ml; Boehringer Mannheim Biochemicals, Inc., Indianapolis, Ind.), and 2 volumes of 100% ethanol. The pellet was collected by centrifugation, washed with 1 volume of ice-cold 70% ethanol, and resuspended in DEPC-treated 1 mM EDTA (pH 7.0).

DNA extraction. DNA extractions were performed by a modification of the method of Fuhrman et al. (7). Cells were collected by filtration onto 0.2-μm-pore-size Millipore GS filters or 0.2-μm-pore-size Durapore filters or by centrifugation and then placed in sterile 2.2-ml tubes. One milliliter of sterile STE (0.1 M sodium chloride, 10 mM Tris-HCl, 1 mM EDTA [pH 8.0] (23) was added, and the sample was stored at −40°C until further processing. The sample was thawed, 0.1 volume of 10% sodium dodecyl sulfate was added, and the sample was placed in boiling water for 2 min. Cell debris was removed by a 10-min centrifugation in a microcentrifuge, and the supernatant recovered. The cell debris pellet was reextracted, and the supernatants from both extractions were combined and precipitated with 0.1 volume of 3 M sodium acetate (pH 5.0) and 2 volumes of 100% ethanol at −40°C overnight. The DNA was collected by centrifugation for 10 min, and the pellet was washed with 1 volume of ice-cold 70% ethanol. The DNA was resuspended in sterile deionized water. To determine the need for further purification, certain samples were also phenol-chloroform extracted after the 70% ethanol wash and reprecipitated with 100% ethanol.

Production of the DNA standard curve. For producing the DNA standard curve, the gene of interest was excised from plasmid pXYL1 (Fig. 1) for *xylE* or from pLC1 (21) for *rbcL*, using the appropriate restriction enzymes. These genes were purified by low-melting-point agarose gel electrophoresis and excision of the appropriate band. The DNA fragment was precipitated and then quantitated by using Hoechst 33258 fluorochrome and fluorometry (19). DNA was then dot blotted onto a charged nylon membrane (Zetaprobe; Bio-Rad, Richmond, Calif.) in decreasing concentrations for use as a standard in DNA quantitation.

RNA and DNA dot blotting and probing. All RNA extracts were divided into three aliquots. One aliquot was left untreated, while the other two were enzyme digested with either RNase or RNase-free DNase (RQ1 DNase; Promega), dot blotted, and probed with antisense (AS) and sense(s) single-stranded RNA probes at 55°C. For DNA, the sample was digested only with RQ1 DNase. The DNA was then dot blotted, fixed to a charged nylon membrane (Zetaprobe), and probed with AS and S RNA gene probes at 42°C as described by Frischer et al. (6). All washings were performed at 65°C as previously described (6). RNA and DNA levels were quantitated by radioactive counting, using a radioanalytic imaging system (β-scanner; Ambis Systems, Inc., San Diego, Calif.), and comparison with RNA and DNA standard curves for the appropriate gene (i.e., *xylE* or *rbcL*).

Synthesis of RNA gene probes and production of the mRNA standard curve. For production of AS and S *xylE* RNA probes, *xylE* was subcloned from plasmid pEPA53 into the riboprobe pGEM3Z to create pXYL1 as shown in Fig. 1. Plasmid pXYL1 was then cut with a restriction enzyme downstream of the RNA polymerase promoter and the *xylE* gene (Fig. 1; *Bam*HI for AS probe, *Pst*I for S probe). The linearized plasmid was then used to synthesize either AS or S RNA probes labeled with ^{35}S-UTP (21). Unlabeled *xylE* mRNA was also synthesized in vitro from this same con-

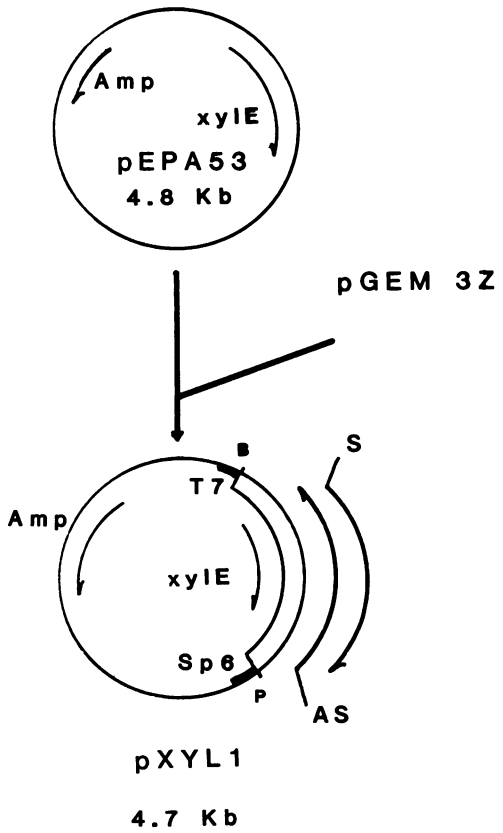

FIG. 1. Plasmid pXYL1, used in this study to produce AS and S probes for the target gene *xylE*. Abbreviations: B, *Bam*HI; P, *Pst*I; Amp, ampicillin.

struct and used as the standard for mRNA quantitation. *rbcL* gene probes were synthesized from plasmid pLC1 as described by Pichard and Paul (21).

Measurement of C2,3O activity. WJT-1C cells in culture or added to seawater were assayed for C2,3O activity by the method of Gibson (8). Cells were harvested by filtration, using 0.2-μm-pore-size Millipore GS filters. The filters were placed in acid-washed plastic snap-cap vials containing 2.0 ml of 0.05 M KH_2PO_4/K_2HPO_4 (pH 7.5)–10% acetone buffer and placed on ice. Cells were then sonicated with a Virsonic cell disrupter (18.5-mm-diameter probe; The Virtis Company, Gardiner, N.Y.) at 60% maximum power on ice for six 30-s pulses with 15 s between pulses. The extract buffer was collected, the vials were washed with an additional 1.0 ml of buffer, and the combined extracts were centrifuged for 10 min. The supernatant was collected, and enzyme activity was determined spectrophotometrically and defined as in the method of Gibson (8). Total protein concentration in the extracts was determined by the method of Lowry et al. (13) with crystalline bovine serum albumin (fraction V; Sigma) as the standard.

Determination of RNA and DNA precipitation efficiency. The efficiency of the precipitation steps in the RNA and DNA extraction protocols was examined by using both in vitro-transcribed *rbcL* mRNA and gel-purified *rbcL* DNA. To examine RNA precipitation efficiency, *rbcL* mRNA was initially added to 1.0 ml of the GIPS reagent, containing 1 μg of tRNA, at decreasing concentrations ranging from 10 to 0.01 ng. The RNA precipitation was done as outlined in the RNA extraction protocol. For determining DNA precipitation efficiencies, *rbcL* DNA was added to 1.0 ml of STE (pH 8.0), containing 1 μg of calf thymus DNA, at the same concentrations as those for mRNA indicated above. The DNA precipitation was performed as in the DNA extraction protocol. The precipitated mRNA and DNA were dot blotted, probed, and compared with the same quantities of mRNA and DNA directly dot blotted.

RESULTS

Efficiency of RNA and DNA extraction. To determine the efficiency of the isolation technique in extracting and recovering RNA, RNA was extracted from *E. coli* B cells grown in minimal medium (Table 2) and compared with the biochemical composition of *E. coli* B cells with a μ of 1.5 doublings per h (4). *E. coli* B cells were doubling every 34 min (μ = 1.76 doublings per h). The RNA extraction efficiency was directly related to the type of filter used to concentrate cells. For cells filtered onto GF/F filters, only 11% of the RNA was recovered, based upon a theoretical yield of RNA for *E. coli* B/r cells. This indicates that the glass fiber GF/F filters bound a substantial portion of the extracted RNA, probably because of the reactivity of glass to nucleic acids. In contrast, the Durapore filters, with low binding capacity, were 70% efficient at recovering total RNA. Both the GF/F filters and the Durapore filters were evaluated for use in detecting *rbcL* gene expression in environmental samples collected from the Gulf of Mexico. The GF/F and Durapore filters retained equivalent amounts of phytoplankton (0.081 ± 0.007 and 0.085 ± 0.002 μg of chlorophyll *a* per liter, respectively). However, phytoplankton RNA extracts (300 ml of offshore seawater) yielded no detectable RNA for the GF/F filters and 5.604 ± 0.822 ng of *rbcL* mRNA per ml for the Durapore filters (data not shown). A previous study by Pichard and Paul (21) showed that the GF/F filters could be used if larger volumes (liters) of water were filtered and dot blots were enhanced with an autoradiographic enhancer. Here the Durapore filters were found well suited for use in detecting and quantitating gene expression in the environment.

Since RNA could be lost during precipitation, the efficiency of the isopropanol and ethanol precipitation steps was examined. The efficiency ranged from 80 to 96%, with an

TABLE 2. Efficiency of RNA extraction from *E. coli* B cells filtered onto various filter types, as determined by spectrophotometry

Filter type (pore size)	Avg A_{260} ± SE	Avg A_{260}/A_{280} ± SE	Measured RNA (μg)[a]	Expected RNA (μg)[b]	% Recovery
Whatman GF/F (0.7 μm)	0.071 ± 0.020	1.885 ± 0.021	2.86	26.25	11
Durapore (0.45 μm)	0.459 ± 0.015	1.925 ± 0.035	18.38	26.25	70

[a] One A_{260} unit equals 40 μg of RNA per ml (23). Samples were assayed in 1-ml quartz cuvettes.
[b] Expected RNA yield (4) calculated for 5.0 ml of mid-log-phase (A_{600} = 0.5) cells at (7.0 ± 0.56) × 10^7 cells per ml.

TABLE 3. DNA determinations for DNA extractions from WJT-1C(pLV1013)[a]

Phenol-$CHCl_3$ extraction	Filter type (pore size)	Avg amt (ng) of DNA extracted[b]
No	GF/F	303 ± 22
	GS (0.22 μm)	594 ± 0
Yes	GF/F	8 ± 0
	GS (0.22 μm)	128 ± 0

[a] Cells were cultured at 27°C, and mid-log-phase (A_{600} = 0.5) cells were filtered and extracted for DNA.

[b] DNA determinations were performed in duplicate using a DNA fluorochrome (Hoechst 33258) and fluorometry (19).

average of 91% ± 7%, over a range of *rbcL* mRNA concentrations (0.01 to 10 ng/ml; data not shown).

A comparison of filters (GF/F and Millipore GS) for use in recovering DNA from *Vibrio* sp. strain WJT-1C containing plasmid pLV1013 appears in Table 3. Phenol-chloroform extraction of the DNA resulted in poor yields, as did the use of the GF/F filters. An alternative filter type, Millipore Durapore, was also examined for use in DNA extractions with a cyanobacterial species, *Synechococcus* sp. strain PCC 6301. DNA extraction yielded an average of 275 ng of DNA for 3.1×10^7 cells irrespective of the method used to collect the cells (i.e., filtration or centrifugation). The average DNA content per cell was 8.9 fg of DNA per cell, which is comparable to DNA-per-cell determinations obtained by flow cytometry for the same organism, assuming two genome equivalents per cell (3).

As for RNA extractions, the efficiency of DNA precipitation was examined with gel-purified *rbcL* DNA. DNA precipitation efficiencies ranged from 78 to 99% ($\bar{x}$ = 90% ± 9%) over a range of *rbcL* DNA concentrations (0.01 to 10 ng/ml; data not shown).

Detection of *xylE* gene expression. The standard curves for *xylE* mRNA and DNA as quantitated by Ambis β-scanning appear in Fig. 2. Similar standard curves were used to quantify *xylE* mRNA and DNA in the time course *xylE* expression experiment (see Fig. 5).

Figures 3 and 4 show the results of detection of *xylE* gene expression in *Vibrio* sp. strain WJT-1C(pLV1013) in culture and when added to nonsterile seawater. An equivalent number of mid-log-phase cells were harvested from both cultures and seawater microcosms for determination of C2,3O activity and RNA and DNA analysis. Cells grown at 28°C had low levels of *xylE* mRNA and C2,3O activity, while cells grown at 40°C exhibited higher levels of both *xylE* mRNA and C2,3O activity. Cells grown at 28 or 40°C had comparable levels of *xylE* DNA, suggesting that changes in *xylE* gene expression levels were not caused by changes in *xylE* gene dose and that gene dose is a valuable parameter against which to normalize mRNA levels.

Figure 5 shows *xylE* gene expression and C2,3O activity of WJT-1C cells grown at the nonpermissive temperature and then incubated at the induction temperature (40°C). The level of *xylE* mRNA was greatest for the first hour at 40°C, with a net synthesis rate of 5 ng of mRNA per ml per h, while little or no C2,3O activity was detected. mRNA levels then declined over the next hour at a net rate of 3 ng/ml/h before leveling off at 2 ng of *xylE* mRNA per ml. During this second hour of incubation C2,3O activity increased by approximately 40-fold, with the appearance of C2,3O activity lagging behind the appearance of *xylE* mRNA by about an hour. Once the *xylE* mRNA level reached a steady state, the

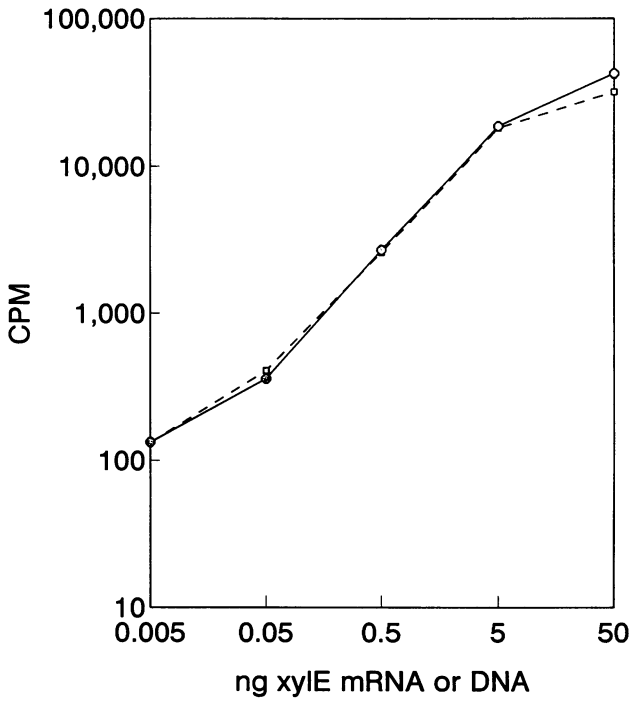

FIG. 2. *xylE* mRNA (solid line) and DNA (dashed line) standard curves, generated from dot blots appearing in Fig. 3, for use in quantitation of cell mRNA and DNA (28).

C2,3O specific activity continued to rise, reaching a maximum of 120 U/mg of protein. Upon reduction of temperature, the *xylE* mRNA content fell to zero and C2,3O specific activity declined to a level of 40 U/mg of protein. The *xylE* DNA content fell within the first hour at 40°C and then began to increase over the rest of the incubation period. However, cell growth followed a different pattern than *xylE* DNA replication, exhibiting a pattern of biphasic growth. Cells grew rapidly within the first hour of incubation at 40°C, increasing their concentration by more than an order of magnitude, before entering a reduced phase of growth over the rest of the incubation. Because the increase in *xylE* DNA was less than the cell growth rate, *xylE* DNA content per cell was actually decreasing over the first hour of the incubation period before a lower *xylE* gene content per cell was established.

DISCUSSION

Changes in levels of target mRNAs in a sample may be caused by variations in transcriptional activity, specific gene copy, cell population size, or a combination of these factors. Therefore, it is necessary to normalize any mRNA signal to some cellular constituent that acts conservatively with growth and biomass. A problem with current environmental gene expression studies is finding a cellular constituent against which to normalize the mRNA level. A standard practice with pure cultures is to use 16S or 23S rRNA as an internal standard to accomplish this task since it is coextracted with the mRNA. However, in the environment, this is not possible because the relationship between the gene of interest and the total rRNA content (16S or 23S) is not known. Our technique eliminates this problem by providing another internal cellular constituent that is specifically asso-

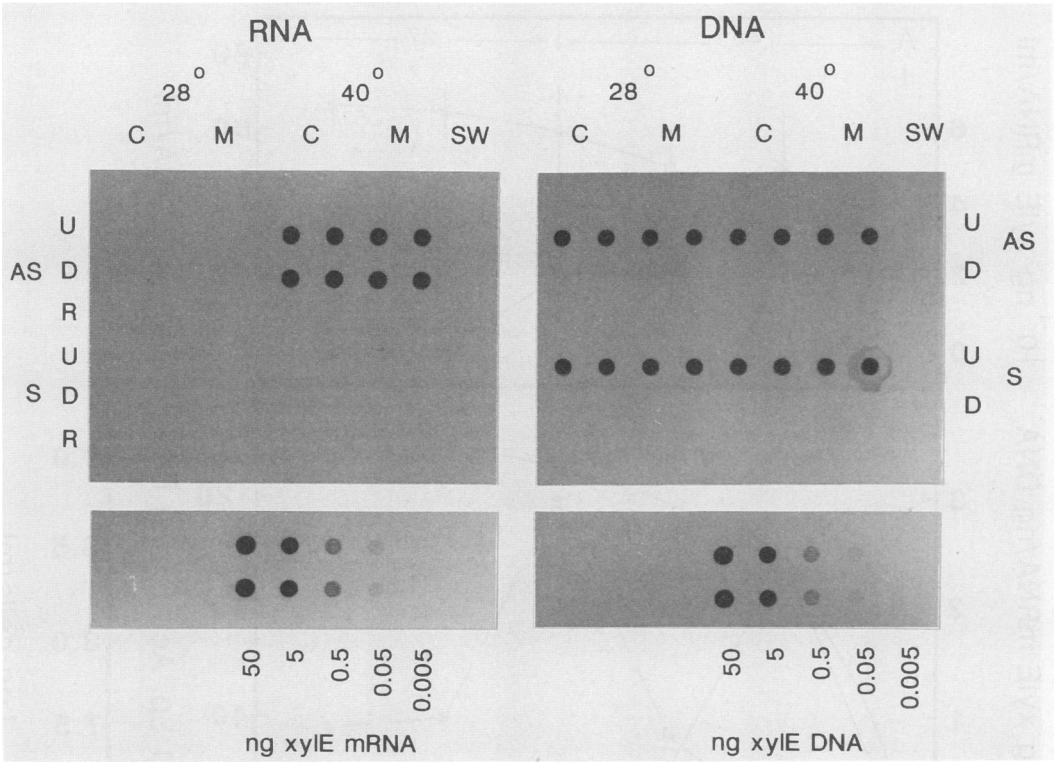

FIG. 3. Detection of *xylE* mRNA and DNA in *Vibrio* sp. strain WJT-1C(pLV1013) from culture and added to seawater. Cells were extracted either from culture (C) or from seawater microcosms (M). A background seawater sample (SW), to which no cells were added, was extracted for RNA and DNA. The samples were probed with the AS or S *xylE* gene probe. The dots in the row labeled U were undigested, whereas those in the rows labeled D and R were digested with DNase and RNase, respectively. *xylE* mRNA and DNA standards are also presented.

ciated with the mRNA being detected, the DNA. The target gene DNA provides a convenient normalizing factor for determining variations in mRNA levels and at the same time corrects for changes in population size.

This method accounts for gene abundance by isolation of target DNA from the same sample by a modification of the method of Fuhrman et al. (7). The method yielded maximal mRNA and DNA recoveries and gave a genomic DNA content for a photoautotrophic picoplankter, *Synechococcus* sp. strain PCC 6301, consistent with a published DNA content for this organism (3). It appears that eliminating the phenol extraction of DNA apparently enables near 100% of the DNA to be recovered. This is important for quantitative studies of changes of absolute copies of DNA. Both the mRNA and DNA extraction methods have been combined with single-stranded AS and S RNA gene probing to quantitatively detect gene expression as mRNA per gene dose.

Proper selection of filters was critical for working with nucleic acids. The use of Durapore filters for collection of cells by filtration enabled increased recoveries of target mRNAs compared with the use of GF/F filters and resulted in a significant improvement over results obtained with our previously published method (21). Total RNA recoveries, compared with theoretical RNA yields previously published (4), are probably overestimates since cells were growing at slightly faster rates. Cells can be expected to have a higher total RNA content at increased growth rates (4). The Durapore filters enabled detection of expression of the *rbcL* gene in natural phytoplankton populations, in which this gene is

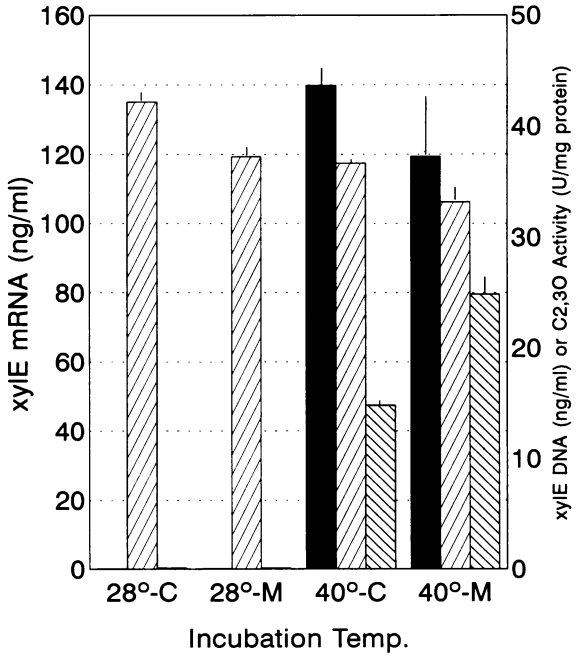

FIG. 4. *xylE* expression in *Vibrio* sp. strain WJT-1C(pLV1013) in culture (C) and seawater microcosms (M), as shown in Fig. 3. Cells for both RNA and DNA extraction were collected by filtration onto 0.2-μm-pore-size Durapore filters. *xylE* mRNA (■) and DNA (▨) and C2,3O activity (▧) are indicated.

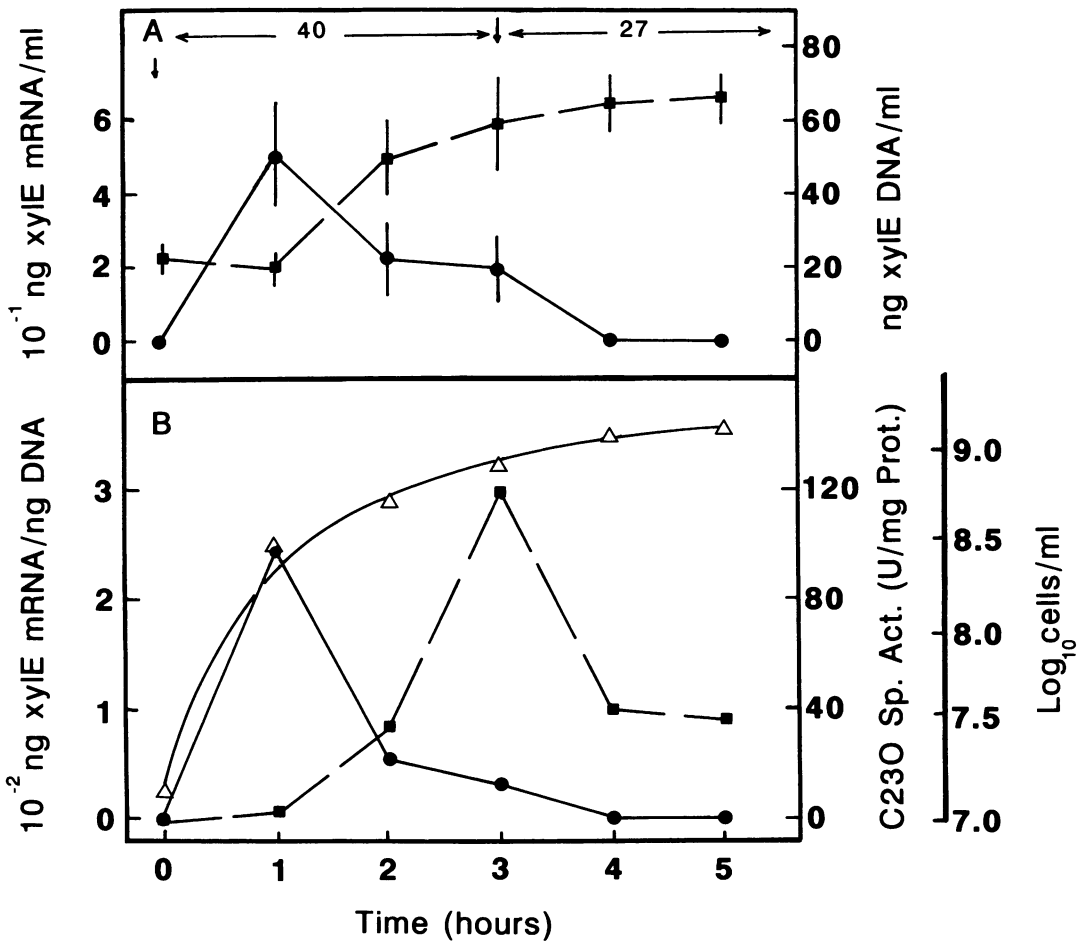

FIG. 5. Kinetics of *xylE* expression in Vibrio sp. strain WJT-1C(pLV1013) in culture. (A) Level of *xylE* mRNA (solid circles) and *xylE* gene dose (solid squares). At time zero the culture was induced for *xylE* expression by elevating the temperature to 40°C. The temperature was returned to 27°C at the 3rd hour. (B) Relationship between *xylE* mRNA/*xylE* DNA (solid circles), C2,3O specific activity (solid squares), and cell growth (open triangles). Cells for RNA extraction were collected by filtration onto Whatman GF/F filters, and for DNA extraction they were collected by filtration onto 0.2-μm-pore-size Millipore GS filters.

abundant and transcriptionally active (21). We intend to study the regulation of RuBPCase by this method.

We have used the RNA-DNA method to monitor *xylE* gene expression by WJT-1C(pLV1013) at 10^6 cells per ml in simulations of the marine environment. Our method was previously found to detect target bacteria, expressing the target gene *nptII*, at a concentration of 10^4 cells per ml of seawater (21) and may even be able to detect gene expression at lower cell concentrations with Durapore filters, although this hypothesis was not tested.

To investigate the relationship between *xylE* mRNA and C2,3O activity, WJT-1C was examined in culture for the appearance of both *xylE* mRNA synthesis and C2,3O activity over an induction period of 3 h. *xylE* mRNA was synthesized first, followed by the appearance of C2,3O activity only after the maximum *xylE* mRNA level was reached. Similar delays in appearance of enzyme activity have been observed for nitrate reductase (NR) in a variety of plants. NR mRNA accumulated rapidly and then declined, while NR activity increased (9, 15). This lag between mRNA and protein synthesis occurs because newly synthesized transcripts must compete with transcripts undergoing translation for the cellular ribosome pool (11).

While detection of low levels of target mRNAs may be indicative of a less transcriptionally active gene, it is important to realize that conservation of nucleotide sequence between gene probe and target will also determine the hybridization efficiency and thus the level of detection of the target. In a heterogeneous population of microorganisms, such as is common in the environment, a myriad of related heterologous target sequences, each with their own probe hybridization efficiency, may exist. In such a case, this method may underestimate the total activity of a target genotype since relatively high stringencies of hybridization were employed. This is why we have restricted our studies of endogenous genes to those that are highly conserved, such as *rbcL*.

The procedure described herein should be applicable to detection and quantification of gene expression, as mRNA per gene dose, by microorganisms released into the environment for bioremediation purposes. The method also provides for the detection of expression of any conserved gene occurring in the indigenous aquatic microbial flora.

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