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Gene Transfer in Marine Water Column and Sediment Microcosms by Natural Plasmid Transformation

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We investigated the possibility for natural transformation in the marine environment by using broad-host-range plasmid multimers and a high-frequency-of-transformation (HFT) *Vibrio* strain as the recipient. Water and sediment samples were taken from Tampa Bay, the eastern Gulf of Mexico, the Florida Shelf near Miami, and the Bahamas Bank. In water column microcosms, transformation frequencies ranged from 1.7×10^{-6} to 2.7×10^{-10} transformants per recipient, with highest frequencies occurring when low levels of nutrients (peptone and yeast extract) were added. The presence of the ambient community either reduced transformation frequency by an order of magnitude or had no effect. In sterile sediments, nutrient additions had no consistent effect on transformation, with transfer frequencies similar to those observed in the water column. Transformation was not observed in any sediment experiment when the ambient microbial community was present. These findings are the first report of natural plasmid transformation in seawater and in the presence of the ambient microbial community. This process may be a mechanism for the acquisition of small, nonconjugative plasmids, which are commonly found in aquatic bacteria. Our data also suggest that natural transformation may be more likely to occur in the water column than in native marine sediments, contradicting prior conclusions based on studies with sterile sediments.

Plasmids are commonly found in bacteria isolated from marine, estuarine, and freshwater environments, with frequencies of plasmid incidence reported at 23 to 46% (3, 10, 13, 27–29, 39). Plasmids are known to occur in antibiotic-resistant marine bacteria (30), and it is thought that the transfer of such plasmids is responsible for the spread of antibiotic resistance among bacteria in the environment (5). However, most of the plasmids found in environmental isolates are too small (<20 kb) to encode genes for self-transmission (12).

Plasmid transfer, although most often associated with conjugation, can also be accomplished by transformation and transduction. Some of the small, naturally occurring nonconjugative plasmids of environmental origin have been transferred by natural transformation (25). Marine bacterial isolates that were naturally transformable with broad-host-range plasmids have been recently described (8, 11).

Currently, there is considerable interest in bacterial gene transfer in aquatic and terrestrial environments (4, 19, 26, 39). Microbial gene transfer mechanisms may have evolved as a means for bacteria to adapt to changing environments and may represent a normal function of bacteria in aquatic and terrestrial ecosystems. The use of genetically engineered microorganisms in the environment and the spread of antibiotic resistances resulting from the use of antibiotics in medicine and agriculture (5) may result in what we term "genetic pollution." Genetic pollution is the introduction of new genetic material or the transfer of genes in the environment resulting from or related to anthropogenic activities. In contrast to other forms of pollution, genetic pollution, once established in a component (i.e., recipient) of an ecosystem, has the capability of self-propagation.

The potential for natural transformation to occur in aquatic environments has not been studied extensively. What few studies have been performed have used bacteria of terrestrial origin (14, 15) and/or chromosomal DNA markers (37) in sediment simulations. We have recently isolated high-frequency-of-transformation (HFT) strains of an estuarine *Vibrio* species (8) that are naturally transformable with a variety of broad-host-range plasmids. These cells possess a unique colony morphology that allows enumeration of recipients against an ambient background flora. Transformants can be easily verified by colony hybridization with probes for the transforming DNA (8, 11). This system is appropriate for the study of transformation in both freshwater and marine environments, because the HFT strains grow equally well in freshwater or marine media (7a). In this report, we describe the use of this system to study the effect of nutrients and the ambient microbial community on natural plasmid transformation in marine water column and sediment microcosms.

MATERIALS AND METHODS

Strains and transforming DNA. The HFT *Vibrio* strain WJT-1C (8) was used as the recipient in all studies. The broad-host-range plasmid pQSR50 (17) was used as transforming DNA. Plasmid DNA and plasmid multimer preparations were performed as described previously (8, 11). Plasmid multimers have been shown to transform at an approximately 10-fold-greater frequency than plasmid monomers (7a).

Sampling sites. Water samples for water column microcosm studies were collected from Bayboro Harbor, St. Petersburg, Fla., the Gulf of Mexico (24°49.94′N, 85°20.00′W), and the Northwest Providence Channel, Bahamas (26°07.90′N, 78°32.50′W). Sediments were collected from North Shore Beach, St. Petersburg, Fla., the Gulf of Mexico near the mouth of Tampa Bay (28°33.89′N, 82°55.03′W), and near Miami (25°45.10′N, 80°04.94′W). Samples for sediment-water column combination microcosms were taken near a coral reef at Joulter's Cay, Bahamas.

Water column transformation assays. A 25-ml overnight

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culture of WJT-1C grown in ASWJP + PY (21) was harvested by centrifugation and resuspended in 20 ml of sterile seawater medium lacking an organic carbon source (ASWJP). A 1-ml (for estuarine microcosms) or 0.5-ml (for Gulf of Mexico and Bahamas microcosms) amount of the cell suspension was added to 24 or 24.5 ml, respectively, of the seawater sample to be investigated. Seawater was either autoclaved and sterile filtered (ASF) or used directly (within 2 h of collection) and added to 50-ml disposable centrifuge tubes. Nutrients were added to some treatments in the form of sterile-filtered solutions of peptone (P) and yeast extract (Y) for final concentrations of 5 mg of P per ml and 1 mg of Y per ml for the estuarine experiment, 0.2 mg of P per ml and 0.04 mg of Y per ml for the Gulf of Mexico experiment, and for the Bahamas experiment, 0.1 mg of P per ml and 0.02 mg of Y per ml (low nutrients) and 1 mg of P per ml and 0.2 mg of Y per ml (high nutrients). Five micrograms of transforming DNA (pQSR50 multimers) was added, and the mixtures were incubated for 10 to 24 h on a gyratory shaker at 1 to 3 rpm at 25 to 30°C. The cells were harvested by either centrifugation or filtration onto a sterile 47-mm Nuclepore filter. The pellets or filters were placed in 5.0 ml of sterile ASWJP, and the cells were resuspended by vortexing for 1 to 2 min. Aliquots of the suspension were diluted and plated in ASWJP + PY for enumeration of total CFU and in ASWJP + PY containing 500 μg of kanamycin per ml, 1,000 μg of streptomycin per ml, and 5×10^{-6} M amphotericin B to detect transformants. The unique colony morphology of WJT-1C allowed enumeration of recipients on nonselective plates in the presence of the ambient population. Transformants in sterile-seawater experiments were enumerated directly on antibiotic plates (spontaneous mutation to resistance to kanamycin and streptomycin has never been observed for WJT-1C). Presumptive transformants from all experiments in the presence of the ambient community were verified by colony hybridization of antibiotic plates (8) using the neomycin-kanamycin phosphotransferase gene (*nptII*) of pQSR50 as a probe. Filters were hybridized with ^{35}S-RNA probes as described previously (8).

Sediment transformation assays. Sediment column transformation assays (11, 37) were performed with estuarine and Gulf of Mexico sediments, whereas transformation assays were performed in sediment plugs for Miami samples. These methods were found to yield equivalent results (data not shown). For sediment columns, 3 cm^3 of freshly collected sediment or autoclaved sediment was used to load columns essentially by the method of Jeffrey et al. (11). When sterile sediment was employed, it was washed twice with sterile ASWJP and charged with 3 ml of 5-μg/ml calf thymus DNA for 1 h to saturate binding sites that had endogenous nucleic acids destroyed by autoclaving (37). The WJT-1C recipient was grown as described above, harvested, and resuspended in 1/10 the original volume of ASWJP, and the cells were added as previously described (11). The columns were incubated for 16 to 24 h at 25 to 28°C. The columns were aseptically dismantled, and the sediment was resuspended in 5.0 ml of sterile ASWJP by 2 min of vigorous vortexing. The overlaying fluid was immediately plated as described above.

For sediment plug transformation assays, 3 cm^3 of autoclaved or fresh sediment was added to a sterile 15-cm^3 disposable conical centrifuge tube. Recipient cells (30-ml culture resuspended in 100 μl), transforming DNA (15 μg), and, in some treatments, nutrients (5 mg of P, 1 mg of Y), were added each in 100-μl volumes, and the sediment was mixed with a sterile pipette. The plugs were incubated overnight and resuspended in 5.0 ml of sterile ASWJP. This suspension was prepared for plating as in the sediment column assays, and presumptive transformants were verified by colony hybridization as described previously (8).

Sediment-water column combination transformation assays. To determine the effect of sediments on water column transformation, transformation assays were performed in 25.0 ml of seawater, 25.0 ml of seawater containing 3 cm^3 of sediment, and 3-cm^3 sediment plugs. Recipient cells were grown as described previously, harvested, and resuspended in 1/10 volume ASWJP, and 0.5 ml of cells was added to each treatment. Five micrograms of transforming DNA was added, and the mixtures were incubated overnight at 25 to 28°C. The cells were harvested by either filtering directly and resuspending the filter in 5.0 ml of ASWJP or adding 5.0 ml of ASWJP to the sediment directly. The sediment and filters were vortexed vigorously for 2 min, and the liquid was plated as described above.

Transfer to the indigenous flora. In most of the experiments performed above, at least one sample received the transforming DNA and no WJT-1C recipients, while a replicate sample received no recipients and calf thymus DNA. Samples were plated as described above, and the antibiotic-resistant colonies were probed for the presence of the *nptII* gene. Liquid enrichments were also made of transformation assays and cells were extracted and probed as previously described (11).

RESULTS

Water column microcosms: effect of nutrients. Addition of nutrients (P and Y) to water column microcosms stimulated growth of both the ambient population and the WJT-1C recipients in all treatments (Fig. 1). The total number of CFU (enumerated on nonselective media) was proportional to the amounts of P and Y added, as can be seen for the Bahamas surface-water experiment (Fig. 1C). The addition of nutrients also resulted in greater numbers of transformants in every experiment (Fig. 1). The least effect of nutrient addition was observed for the eutrophic estuarine environment, where the total number of transformants in the nutrient-amended sample was only twice that of the unamended sample. The ambient levels of dissolved nutrients in the estuarine sample probably allowed transformation as efficiently as in the nutrient-amended samples.

In four of six experiments, an increase in transformation frequency in nutrient-amended samples was observed (Table 1). Low levels of nutrients (100 μg of P per ml, 20 μg of Y per ml) stimulated transformation in the presence or absence of the ambient community in Bahamian samples, whereas high levels of nutrients resulted in transfer frequencies below those found in the unamended samples. Thus, low levels of nutrients apparently stimulated transformation better than high levels of nutrients.

Water column transformation experiments: effect of the ambient population. The presence of the ambient population inhibited gene transfer in two of three experiments (estuarine and Bahamas water experiments; Fig. 1 and Table 1). The ambient population had no effect on transfer in the oligotrophic waters of the Gulf of Mexico (Table 1). This may have been caused by the numerical excess of the recipient compared with the ambient population in these waters. In all other experiments, the presence of ambient community resulted in approximately a one-log decrease in transformation frequency (Table 1), with an exception in the nutrient-amended estuarine treatment.

Sediment microcosm experiments. Transformation oc-

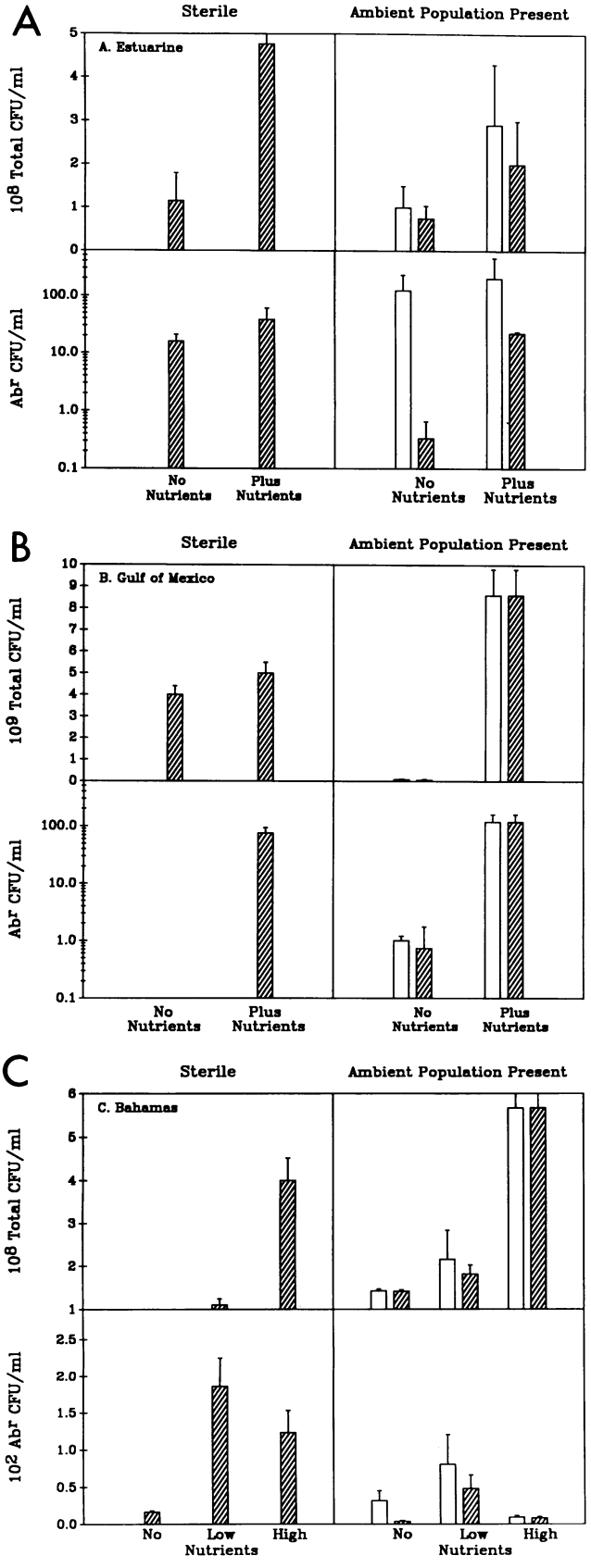

FIG. 1. Natural plasmid transformation in water column microcosms sampled from Tampa Bay (A), oligotrophic surface water from the Gulf of Mexico (B), and surface water from Northwest Providence channel, Bahamas (C). Shown in the top panels of each set of graphs are CFU enumerated on ASWJP, and shown in the bottom panels of each set of graphs are the antibiotic-resistant CFU. The open bars indicate the total bacteria present (WJT-1C recipient plus the ambient population). The hatched bars indicate WJT-1C recipients only. Experiments for the left panels were all performed in sterile microcosms, and experiments for the right panels were performed in the presence of the natural population. No Nutrients indicates that no exogenous nutrients were added, and Plus Nutrients indicates that sterile P and Y were added (see Materials and Methods for details).

TABLE 1. Antibiotic resistance and transformation frequencies in water column microcosm experiments

Environment[a]	Resistance[b] frequency	Transformation[c] frequency	Transformants[d]/Ab^r cells (%)
Estuarine			
ASF	1.33×10^{-7}	1.33×10^{-7}	100
ASF + nut.	8×10^{-8}	8×10^{-8}	100
Raw	1.2×10^{-6}	4.5×10^{-9}	0.3
Raw + nut.	6.8×10^{-7}	1.1×10^{-7}	11.1
Oligotrophic Gulf of Mexico			
ASF	0	0	
ASF + nut.	1.5×10^{-8}	1.5×10^{-8}	100
Raw	1.2×10^{-8}	1.2×10^{-8}	73
Raw + nut.	1.4×10^{-8}	1.4×10^{-8}	100
Oligotrophic Bahamas			
ASF	8×10^{-7}	8×10^{-7}	100
ASF + low nut.	1.7×10^{-6}	1.7×10^{-6}	100
ASF + high nut.	3.1×10^{-7}	3.1×10^{-7}	100
Raw	6.3×10^{-7}	7.6×10^{-8}	11.9
Raw + low nut.	5.8×10^{-7}	4.7×10^{-7}	60.2
Raw + high nut.	1.0×10^{-8}	1.5×10^{-8}	91.2

[a] ASF, autoclaved and sterile filtered; nut., sample amended with P and Y (see Materials and Methods); raw, nonsterile sediment with ambient community present.
[b] Frequency of appearance of resistant bacteria (total antibiotic-resistant CFU/total CFU).
[c] Number of WJT-1C transformants divided by total WJT-1C present.
[d] Number of WJT-1C transformants divided by total antibiotic-resistant (Ab^r) CFU × 100.

curred in sterile-sediment microcosms at frequencies which were not significantly different than those observed in the water column (Table 2 and Fig. 2). The addition of nutrients had no consistent effect on transformation and stimulated transfer in only one of three experiments (estuarine samples). These results imply that transformation was not nutrient limited in the sediments investigated. The greatest effect on transformation was observed in nonsterile sediments. No transformation in the presence of the ambient community in any sediment environment was observed.

Sediment-water column microcosm experiments. To determine the effect of the presence of sediments on gene transfer in the water column, experiments on liquid transformation in the presence of sediments were performed, and results were compared with those for experiments with liquid only and sediment only (Fig. 3 and Table 2). In sterile microcosms, transformation in water column, water plus sediment, and sediment environments was detected, with greatest frequency in the sediment (1.8×10^{-7} compared with $1.2 \times$

TABLE 2. Transformation in sediments and water column-sediment microcosms

Environment[a]	Resistance[b] frequency	Transformation[c] frequency	Transformants[d]/Ab^r cells (%)
Estuarine			
Sterile sediment	0	0	
Sterile sediment + nut.	1.5×10^{-8}	1.5×10^{-8}	100
Raw sediment	3.3×10^{-8}	0	0
Gulf of Mexico Shelf			
Sterile	6.6×10^{-8}	6.6×10^{-8}	100
Sterile + nut.	4×10^{-9}	4×10^{-9}	100
Raw	7×10^{-8}	0	0
Raw + nut.	1.75×10^{-8}	0	0
Miami sediments			
Sterile	5×10^{-8}	5×10^{-8}	100
Sterile + nut.	4.8×10^{-8}	4.8×10^{-8}	100
Raw	4.8×10^{-9}	0	0
Raw + nut.	3.5×10^{-9}	0	0
Joulter's Cay, Bahamas			
Sterile water column	1.3×10^{-8}	1.3×10^{-8}	100
Sterile water column + sediments	1.2×10^{-8}	1.2×10^{-8}	100
Sterile sediments	1.8×10^{-7}	1.8×10^{-7}	100
Raw water column	3.8×10^{-9}	2.7×10^{-10}	6.7
Raw water column + sediments	6.6×10^{-7}	0	0
Raw sediments	4.4×10^{-7}	0	0

[a] nut., sample amended with P and Y (see Materials and Methods); raw, nonsterile sediment with ambient community present.
[b] Frequency of appearance of resistant bacteria (total antibiotic-resistant CFU/total CFU).
[c] Number of WJC-1C transformants divided by total WJC-1C present.
[d] Number of WJC-1C transformants divided by total antibiotic-resistant (Ab^r) CFU × 100.

10^{-8} to 1.3×10^{-8}; Table 2). When the ambient population was present, transfer was observed only in the water column treatment. The presence of nonsterile sediment inhibited the transformation process, even in the water column-plus-sediment treatment (Fig. 3 and Table 2). The number of antibiotic-resistant bacteria in the sediments exceeded that in the water column by nearly two orders of magnitude. It may be that the sediment bacteria outcompeted the transformants for nutrients and/or transforming DNA. In no experiment was transfer to the ambient population observed.

DISCUSSION

The above experiments demonstrate the capability for natural plasmid transformation to occur in marine water column environments in the presence or absence of the ambient microbial community. The frequencies of transfer observed ranged from 1.7×10^{-6} to 2.7×10^{-10} transformants per recipient and were generally several orders of magnitude below that observed for filter transformation assays (10^{-6} to 2.5×10^{-4} [8]). There are few reports of transformation in liquid, and when observed, it has been shown to be less efficient than transfer on filters or the surfaces of agar plates (34–36). For example, a soil strain of *Pseudomonas stutzeri* could not be transformed in liquid

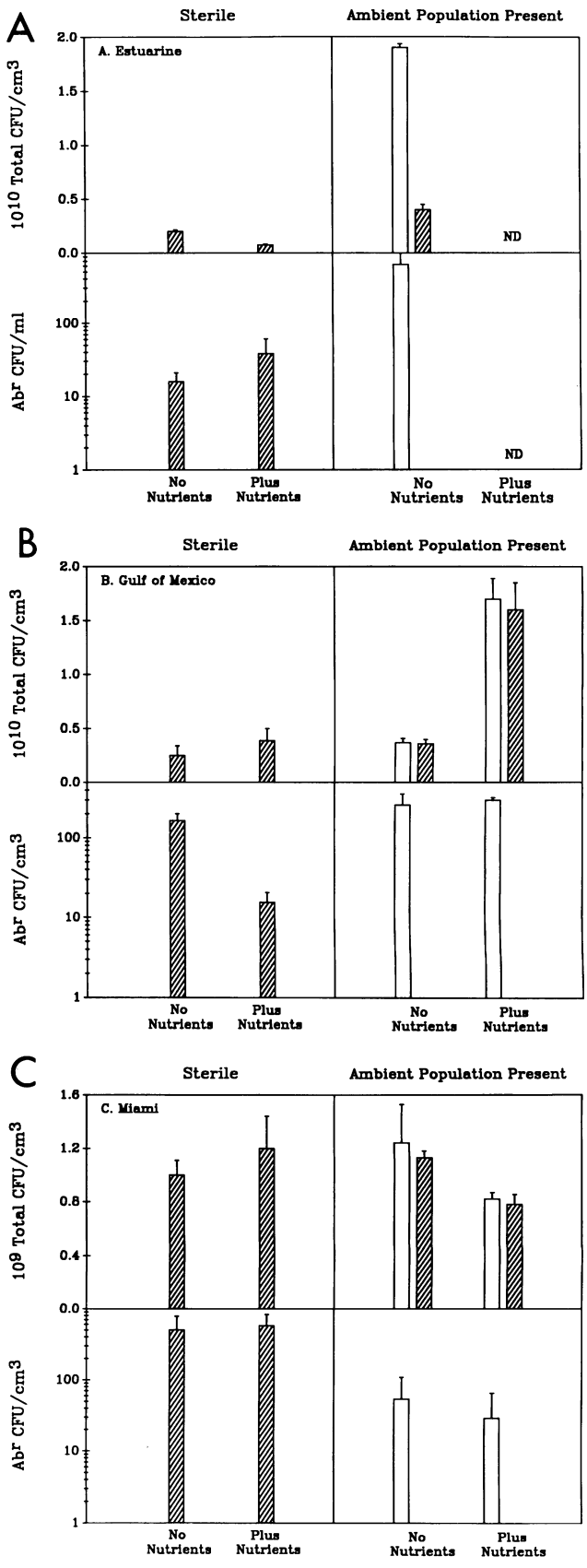

FIG. 2. Natural plasmid transformation in sediment microcosms sampled from Tampa Bay (A), the Gulf of Mexico (B), and the Florida Shelf near Miami (C). Symbols and organization of panels are as in legend for Fig. 1. ND, not determined.

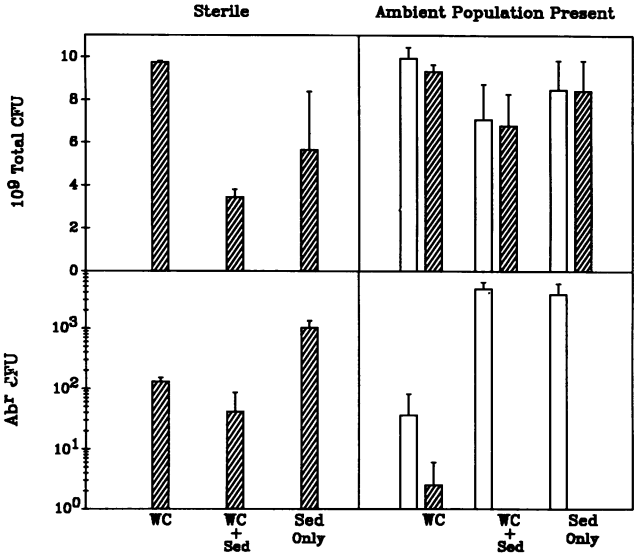

FIG. 3. Effect of sediments on water column transformation in samples taken near a coral reef in Joulter's Cay, Bahamas. WC, water column transformation assay; WC + Sed, water column containing 3 cm^3 of sediment; Sed Only, transformation in sediment plugs (see text for details). Total CFU and antibiotic-resistant (Ab^r) CFU values are per entire microcosm. Symbols and organization of panels are as in legend for Fig. 1.

unless glass beads were added (35). The transfer frequency in liquid in the presence of glass beads (10^7 per recipient) was nearly three orders of magnitude below that observed on filters. Similarly, *P. stutzeri* Zobell, a marine isolate, could not be transformed in liquid (36). Transformation on filters (36) and in sediment columns (37) was observed with this organism. Rochelle et al. (25) observed frequencies of transformation in liquid similar to those for matings on agar surfaces when whole cells were used as plasmid donors (10^{-4} to 10^{-5} per recipient). However, when free DNA was used in assays on plates, the frequency of transfer was approximately two orders of magnitude below that observed with donor cells as a source of transforming DNA. Therefore, transformation in water column environments seems more likely to occur when cells are used as DNA donors than with free DNA.

Transformation in liquid has been reported for the photosynthetic cyanobacterium *Agmenellum quadruplicatum* (i.e., *Synechococcus* strain 7002 [7, 32, 33]). As with other studies of transformation in liquid, these experiments were performed in culture media. Our work demonstrates the first report of bacterial transformation in unamended seawater and the first report of bacterial transformation in the presence of the ambient community. As with transduction (18, 20), the presence of the ambient community hampered detection of transformation. In transduction microcosms, the presence of the ambient population resulted in a rapid decrease in the recoveries of transductants, introduced donors, and recipients. However, transduction frequencies were similar, suggesting that the presence of the ambient population hindered only detection of transductants and not the absolute potential for gene transfer by this mechanism (18). In our work, the presence of the ambient community resulted in a decrease in the recipient population in only one of six treatments but resulted in a decreased transformation frequency in four of six treatments (Table 1). It may be that nuclease digestion of DNA (by either dissolved nucleases or cell-associated nucleases [16, 24]) or competition for DNA uptake by the natural population may have resulted in the lower transfer frequencies observed.

In water column experiments, addition of low levels of nutrients resulted in elevated transformation frequencies. The addition of nutrients has previously been shown to stimulate gene transfer in soil microcosms (2, 38, 40). The effect of nutrients in the present study was probably caused in part by physiologic responses of the recipient and by patterns of nutrient utilization of the ambient community. Studies on the induction of competence by the *Vibrio* recipient indicate that late-stationary-phase cells are the most competent (7a). Actively growing (log phase) cells are the least competent. When no nutrient addition was made, cells did not reach their competent state and were likely in a dormant or death phase. The addition of low levels of nutrients as in the Bahamas experiment probably enabled cells to reach a stationary phase rapidly. High levels of nutrients resulted in no more competence than the absence of exogenous nutrients. These observations are consistent with the hypothesis that induction of competence occurs during the switch from exponential growth phase to stationary phase or the change from balanced growth to unbalanced growth. Induction of competence during metabolic shiftdown in the gram-negative *Haemophilus* transformation system has been observed. Transferring *Haemophilus* cells from rich media to minimal media results in competence induction in 100% of the population (31). Maximal competence in *P. stutzeri* Zobell was observed 14 h after putting log-phase cells on agar plates, again consistent with induction of competence corresponding to a metabolic shiftdown (36).

A second possible explanation for the stimulation of transformation by nutrients in the presence of the ambient population is that an alternate substrate for microbial growth is provided in addition to the transforming DNA. This may prolong the survival of the naked transforming DNA.

The ecological significance of our findings with nutrients is that transformation is probably more likely to occur in environments receiving organic inputs than in oligotrophic environments. Thus estuaries, waters receiving sewage or other nutrient-rich effluents, wastewater treatment facilities, and other environments with organic carbon inputs favor transfer by transformation (and probably gene transfer by other mechanisms). In oceanic water column environments, transformation may occur in microenvironments associated with detrital particles, in marine snow, in *Trichodesmium* colonies, in *Sargassum* communities, during transient events such as phytoplankton blooms, or during upwelling events.

The capability for natural plasmid transformation to occur in sterile sediments is important because it demonstrates that this process can occur in this type of environment and at least is not limited by abiotic processes. The first demonstration of the potential for transformation to occur in the environment was in sterile soil by using *Bacillus subtilis* and chromosomal markers (9). This system was later used in sterile marine sand to demonstrate that transforming DNA was protected from nuclease degradation when bound to sediment (1). Further studies indicated that transformation occurred more readily in sterile sand than in solution (14). A similar series of experiments carried out with *P. stutzeri* in sterile sand demonstrated that natural transformation can occur with gram-negative bacteria in sediment microcosms.

Demonstration of transformation in sterile sediments does

not mean that this process can occur in the presence of the ambient population. There have been few reports of natural transformation in nonsterile soil or sediments. Stotsky (38) reported natural plasmid transformation in nonsterile soils at frequencies several orders of magnitude below that found in sterile soil. This research emphasized the importance of the indigenous bacteria in the survival of genetically engineered microorganisms in the environment and in their capability to transfer genes in these environments.

Stewart and Sinigalliano (37) recently reported natural transformation of chromosomal markers in *P. stutzeri* Zobell in nonsterile marine sediments. Transformation was detected as an increase in rifampin-resistant CFU in sediments amended with transforming DNA compared with CFU in sediments with DNase and in no-DNA controls. We have also observed apparent natural plasmid transformation of ambient microbial populations by increases in antibiotic-resistant CFU compared with CFU in DNase controls (23). However, these apparent transformants did not contain the transforming DNA as determined by molecular probing (23). This emphasizes the need for definitive methods for verifying acquisition of the transforming DNA (e.g., gene probes for target DNA sequences) besides enumeration of antibiotic-resistant or auxotrophic CFU. The abundance of antibiotic-resistant organisms in the sediment makes detection of transformants difficult to impossible when chromosomal DNA (or any transforming DNA for which a probe is not available) is employed. In the present study, verification of transformation to recipients was obtained by probing colony lifts (i.e., colony hybridization) of at least one plate that contained putative transformants from each treatment.

One possible reason that transformation was not detected in nonsterile sediments is that the sensitivity of the assay was not sufficient to detect it. Our assay would allow detection of transformation at frequencies as low as 8.3×10^{-11}. The average frequency in sterile sediment was 7×10^{-8}. If the presence of the ambient community lowered the frequency by one or two orders of magnitude, then transformation should have been detected. If the ambient community lowered the frequency by three or more orders of magnitude, transformation would not be detected. Because recipient cells could be easily recovered from nonsterile sediments on enumeration plates, it seems unlikely that cell binding or grazing or viral lysis reduced our ability to detect transformation unless transformants were preferentially affected. Although some sediments contained large numbers of antibiotic-resistant bacteria, other sediments (notably the Miami sample) had relatively low numbers of these bacteria, such that prevalence over transformants or simply overgrowth of transformants by the ambient population seems unlikely in all instances. There may be an abundance of DNA-hydrolyzing bacteria in the sediments or extracellular nucleases that degrade DNA compared with the water column (studies on sterile or artificial sediments would not detect this). Alternatively, native sediments may be sufficiently reduced or contain metabolic poisons (e.g., sulfides) that inhibit transformation. This latter possibility seems likely in view of the fact that 1/10 volume of nonsterile sediment added to a water column microcosm completely inhibited transformation.

No transformation of the ambient population was observed. We have performed similar transformation assays in the absence of the recipient and have not detected transfer to the indigenous community (23). Reasons for lack of detection of transfer may be insensitivity of the assay, inappropriateness of the transforming DNA, low levels of competent cells, and short exposure (i.e., overnight) of the community to the transforming DNA.

The goal of any microcosm system is to simulate the environment. The water column transformation experiments described herein contained 10^7 to 10^8 recipients per ml, one to two orders of magnitude more cells than in the ambient community, all of the same strain. Additionally, the cells employed were from nutrient-rich cultures, unlike the cells in the environment. Lastly, the concentration of DNA employed (0.1 to 0.2 μg/ml) was 10 to 20 times the ambient level of dissolved DNA one might expect to find in such environments (6, 22). The cell and DNA concentrations employed were necessary to detect transformation by the system we employed (i.e., nonhomologous plasmids as transforming DNA and the high-frequency-of-transformation strains). Higher transformation frequencies for studies in which homologous strains were used as DNA donors and recipients have been reported (25). Such strains could occur in the environment at ambient bacterial abundances. Additionally, detection of transformation was limited to cultivable forms. It is well known that environmental isolates often return to a viable but nonculturable state when returned to the environment, such that the true potential for transformation may have been underestimated. Lastly, our system for gene transfer was a homogenous batch system, with defined cell and DNA concentrations. These cell and DNA concentrations might be found in the microenvironments described above. For example, the DNA concentration inside a detrital particle may be high compared with the concentration in the surrounding bulk water, and the particle might be colonized by one or several bacterial species. Biofilm formation in the environment often results in colonies of single species that might provide the prerequisite numbers of recipient and the concentration of homologous DNA necessary for transformation. Currently, there is no technology available to work with such systems in nature.

The significance of our work is the report of an efficient transformation system with identifiable markers for use in freshwater or marine water column and sediment (and potentially soil) environments. Using this system, we report the first instance of natural plasmid transformation in seawater and in the presence of the ambient community in seawater. Our results indicate that there is a greater likelihood of transformation occurring in the water column than in marine sediments.

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