Factors affecting competence in a high frequency of transformation marine *Vibrio*

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Natural plasmid transformation may be a mechanism for the horizontal transfer of non-conjugative plasmids in the marine environment, yet there are few marine model systems available for the study of this process. Using multimers of IncQ/P4 plasmids and a filter transformation assay, we have measured the effects of nutrients, salinity, temperature, as well as the development and maintenance of competence for genetic transformation in the high frequency of transformation (HFT) marine *Vibrio* strain WJT-1C. Transformation frequency was proportional to the amount of DNA used from 0.1 to 1.0 µg DNA and was saturated at concentrations greater than 1.0 µg. Competence began in the early-exponential phase and reached a maximum at the onset of stationary phase. Once attained, competence was maintained in both spent and nutrient-free media for at least 10 d. Thus, the establishment and maintenance of competence was unique compared to previously described transformation systems. Temperatures ranging from 4 to 33 °C had no significant effect on the maximal transformation frequency of WJT-1C, but at 37 °C the transformation frequency was reduced. However, temperature did affect the rate of the transformation process. Salinities in the range 12 to 50‰ had no significant effect on the transformation frequency but transformation frequencies were lower at 6‰ and 63‰. Cells were transformed equally well in nutrient-free media or rich media. The ability of this marine HFT *Vibrio* strain to develop competence under a wide spectrum of conditions and to maintain the competent state indicates that natural plasmid transformation could occur in conditions found in tropical and subtropical estuaries.

Introduction

During the last two decades a considerable amount of evidence has been collected indicating that prokaryotic gene exchange is an important microbial process in aquatic and terrestrial environments. The potential for genetic exchange by conjugation, transduction and natural transformation has been demonstrated in a variety of diverse environments (Graham & Istock, 1978; Trevors & Oddie, 1986; Bale *et al.*, 1987, 1988; Rochelle *et al.*, 1988; VanElsas *et al.*, 1989; Fry & Day, 1990; Henschke & Schmidt, 1990; Paul *et al.*, 1991; Pretorius-Guth, 1990; Saye *et al.*, 1990; Stewart & Sinigalliano, 1990; Klingmuller, 1991). The occurrence of horizontal gene transfer events has been inferred by examining the

distribution and genetic structure of specific phenotypes (e.g. streptomycin resistance) in natural microbial populations (Norelli *et al.*, 1991; Smith *et al.*, 1991). Additionally, gene transfer events from defined donors to indigenous microbial populations in a variety of environmental simulations have been documented (Henschke & Schmidt, 1990; Stewart & Sinigalliano, 1990; Fulthorpe & Wyndham, 1991; Smit *et al.*, 1991).

Natural transformation as a mechanism of genetic transfer in marine environments has not been extensively studied. Transformation is the process by which cells take up and express genes encoded by extracellular DNA (Avery *et al.*, 1944). Natural transformation is distinct from artificial transformation (a widely used technique in molecular biology for the induction of competence in cells by chemical, enzymic or physical means) as being a normal physiological property of certain bacterial species (Smith *et al.*, 1981; Saunders & Saunders, 1988; Stewart, 1989). The presence of high-molecular-mass extracellular DNA (DeFlaun *et al.*, 1987; Paul *et al.*, 1987) and competent bacterial strains (Jeffrey *et al.*, 1990; Rochelle

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Abbreviations: DAPI, 4',6-diamidino-2-phenylindole; HFT, high frequency of transformation.

et al., 1988; Saunders & Saunders, 1988) suggests that this process can occur in aquatic environments.

The transfer of plasmids amongst natural bacterial populations in the environment is of special interest since plasmids may increase the metabolic potential of the indigenous flora (Novick, 1969; Broda, 1979; Foster, 1983; Schutt, 1990). Although the transfer of plasmids amongst bacterial communities is most often associated with conjugation, plasmid transfer can also be accomplished by transformation. Fry & Day (1990) found that 65% of endogenously isolated plasmids from the River Taff, Wales, were less than 10 kb, and therefore too small to encode conjugation genes. Furthermore, one of these small plasmids (pQM17; 7.8 kb), which encoded mercury resistance, could be transferred by natural transformation between *Acinetobacter* strains (Rochelle *et al.*, 1988). Thus, transformation may be a mechanism responsible for the dissemination of small plasmids in aquatic environments.

Few studies have investigated the transformation process in marine organisms. The most extensive transformation studies have focused on three genera, *Streptococcus*, *Haemophilus* and *Bacillus*, and two transformation models have been developed based on these organisms (Smith *et al.*, 1981; Stewart, 1989). However, other organisms seem to vary appreciably from these models (Stewart, 1989). Therefore, in order to understand natural transformation as a genetic exchange mechanism in marine environments, it is necessary to develop a model for natural transformation employing a marine micro-organism.

Recently we have described high frequency of transformation (HFT) marine *Vibrio* strains which can be transformed by plasmid DNA (Jeffrey *et al.*, 1990; Frischer *et al.*, 1990; Paul *et al.*, 1991, 1992). In these investigations we have continued to develop these strains as transformation models to help understand the process of gene transfer in the marine environment.

Methods

Strains and plasmids. Bacterial strains and plasmids used as transforming DNA are listed in Table 1. The HFT *Vibrio* strain WJT-1C (Frischer *et al.*, 1990) was used as the recipient in all studies. The IncQ/P4 broad-host-range non-conjugative plasmids RSF1010, R1162 and pQSR50 were examined for their transforming activity and compared to the previously measured transforming activity of another IncQ/P4 plasmid, pGQ3 (Frischer *et al.*, 1990). RSF1010 and R1162 are believed to be identical (Frey & Bagdasarian, 1989). pGQ3 is a 13.5 kb derivative of pKT230 (an RSF1010 derivative) that contains the *Escherichia coli* thymidine kinase gene (Carlson *et al.*, 1985). pQSR50 is a 14.4 kb, Tn5-containing derivative of the plasmid R1162 (Meyer *et al.*, 1982). After initial experiments the plasmid pQSR50 was chosen for all following investigations.

Culture conditions. In all experiments, *Vibrio* strain WJT-1C was grown in artificial seawater (ASWJP; Paul, 1982) supplemented with peptone and yeast extract (PY; Difco) at 29 °C except where noted.

Unless otherwise specified the concentrations of peptone and yeast were 5 and 1 g l⁻¹, respectively. *E. coli* strains were grown at 37 °C in LB (Sambrook *et al.*, 1989) containing appropriate antibiotics.

Preparation of plasmids as transforming DNA. Plasmid DNA was purified by alkaline lysis as described by Griffith (1988) and further purified by use of a pZ523 plasmid purification column (5' → 3' Inc., West Chester, PA, USA) (Felsenstein, 1988; Zervos *et al.*, 1988). Plasmid preparations enriched in linear or oligomer forms were prepared by cutting to completion at a unique restriction site or by restriction digestion followed by ligation with T4 ligase, respectively (Jeffrey *et al.*, 1990; Frischer *et al.*, 1990). Oligomerization was judged by visualization on a 0.4% (w/v) agarose gel stained with the fluorochrome Hoechst 33258 (DeFlaun & Paul, 1986) as previously described (Frischer *et al.*, 1990).

Transformation assays. The standard filter transformation assay was performed essentially as previously described except that stationary-phase (16–20 h growth period) cells were used, unless noted otherwise (Jeffrey *et al.*, 1990; Frischer *et al.*, 1990). Transformants were enumerated on ASWJP+PY plates containing kanamycin (500 µg ml⁻¹) and streptomycin (1000 µg ml⁻¹). Transformation frequency is reported as the number of antibiotic-resistant c.f.u. per total c.f.u. Where specified, transformation was inhibited by the addition of 100 Kunitz of DNAase I in 4.2 mM-MgCl₂ to filtered cells. All antibiotics and DNAase I were purchased from Sigma.

To determine the time required for transformation, 1 ml of a stationary-phase culture of *Vibrio* WJT-1C (approximately 10⁹ cells) was immobilized onto a 0.2 µm Nucleopore filter and overlaid with 4 µg of transforming DNA as in the standard transformation assay, but at appropriate intervals DNAase I was added to inhibit further transformation.

To investigate the development of competence, early-exponential-phase cultures were used to inoculate fresh media and transformation was assayed as a function of growth phase using the standard assay. Growth was determined by measuring the optical density of the culture at 600 nm. Transformation was determined at discrete growth phases by exposing cells to transforming DNA for 2 h, after which DNAase I was added to the filter. To determine whether transformation was completely inhibited by the DNAase treatment, DNAase was added immediately following addition of transforming DNA to replica treatments. In these controls, no transformants were ever detected. The approximate concentration of cells was 10⁹ cells per filter.

The maintenance of competence in spent media was investigated by allowing a culture of WJT-1C to incubate shaking at 29 °C for 10 d. Competence maintenance under starvation conditions was also determined by incubating washed cells in ASWJP without nutrients under the same conditions for 10 d. In the latter studies the concentration of the culture was maintained at low cell densities (approx. 10⁶ cells ml⁻¹) to simulate naturally occurring bacterial densities and to prevent nutrient scavenging. In both conditions (i.e. spent or starvation media), the culture was sampled daily for 5 d and again after 10 d. Viable counts (total c.f.u. ml⁻¹), direct cell counts and the transformation frequency were determined at all sampling times.

To investigate the effect of temperature on the transformation frequency, cultures of WJT-1C were grown to late-exponential phase (OD₆₀₀ 0.8) at temperatures ranging from 15 to 37 °C and transformed at the same temperatures as used for growth. For transformation assays at 4 °C, cells were grown at 15 °C and then transferred to 4 °C after being filtered.

To determine the effect of salinity, media of various salinities were prepared by dilution of double-strength artificial seawater (2 × ASWJP) with deionized water. The lowest-salinity medium (6‰) was prepared by adding PY to deionized water. Cultures of WJT-1C were then grown and transformed using the standard filter assay at salinities ranging from 6 to 63‰.

Table 1. *Bacteria and plasmids*

Strain or plasmid	Phenotype or description	Source or reference
Strains		
<i>Vibrio</i> WJT-1C	HFT derivative of estuarine isolate DI-9	Frischer <i>et al.</i> (1990)
<i>E. coli</i> RM1208(R1162)	F ⁻ <i>thi lacY thr leuB supE44 ΔtrpE5</i>	Meyer <i>et al.</i> (1982)
<i>E. coli</i> RM1259(pQSR50)	F ⁻ <i>thi lacY thr leuB supE44 ΔtrpE5</i>	Meyer <i>et al.</i> (1982)
<i>E. coli</i> JL3700(RSF1010)	F ⁻ <i>gal thi fhuA endA sbcB15 hsdR4 shdM⁺</i>	Gift of G. Stewart, Univ. S. Florida, Tampa
<i>E. coli</i> JL4062(pGQ3)	HB101	Carlson <i>et al.</i> (1985)
Plasmids		
R1162	IncQ/P-4 Su ^r Sm ^r Mob ⁺	Meyer <i>et al.</i> (1982)
RSF1010	IncQ/P-4 Su ^r Sm ^r Mob ⁺	Frey & Bagdasarjian (1989)
pQSR50 (= R1162::Tn5)	IncQ/P-4 Su ^r Sm ^r Km ^r Mob ⁺	Meyer <i>et al.</i> (1982)
pGQ3	IncQ/P-4 Su ^r Sm ^r Km ^r tdk ⁺ Mob ⁺	Carlson <i>et al.</i> (1985)

The effect of nutrient concentration on transformation was evaluated with the standard assay by growing and transforming cells at nutrient concentrations ranging from ASWJP + 1% (w/v) PY (0.05 g P and 0.01 g Y l⁻¹) to ASWJP + 200% (w/v) PY (10 g P and 2 g Y l⁻¹).

Results

Transforming plasmid DNA

Table 2 shows the results of transformation of the HFT *Vibrio* strain WJT-1C with multimers of four non-conjugative broad-host-range IncQ/P4 plasmids. Multimers of the plasmids RSF1010 and R1162 yielded the highest transformation frequencies, from 4.5 to 9.1 × 10⁻³ transformants per recipient. Transformation of WJT-1C with pQSR50 multimers resulted in transformation frequencies approximately two orders of magnitude lower than did RSF1010 and R1162. pGQ3 multimers had the lowest transforming activity, approximately three orders of magnitude lower than that of pQSR50. Transformation frequencies were compared by analysis of variance and multiple-range testing (Zar, 1984). It was concluded that multimers of RSF1010 and R1162 had identical transforming activities, while pGQ3

Table 2. Transformation frequency of WJT-1C with IncQ/P4 plasmids

Plasmid	Selected markers*	Transformation frequency (±SD)†	No. of trials
RSF1010	Sm	9.1 (2.2) × 10 ^{-3a}	7
R1162	Sm	4.5 (1.8) × 10 ^{-3a}	4
pQSR50	Km, Sm	5.5 (3.9) × 10 ^{-5b}	19
pGQ3	Km, Sm	4.4 (3.2) × 10 ^{-8c}	2

* Km, kanamycin; Sm, streptomycin.

† a, indicates results that are statistically identical (*P* > 0.5); b, indicates significant difference from the transformation frequency of RSF1010 and pGQ3 (0.01 < *P* < 0.001); c, indicates significant difference from the transformation frequency of RSF1010 and pQSR50 (0.05 < *P* < 0.01).

Fig. 1. Autoradiograph of a Southern transfer of pQSR50 plasmid preparations hybridized with ³⁵S-labelled Riboprobe RNA probe pNPTII. A, untreated plasmid preparation; B, linear enrichment; C, oligomer enrichment. Bands 1–5, marked on the autoradiograph, correspond to: (1) linear dimer, (2) covalently closed relaxed monomer, (3) covalently closed oligomer, (4) linear, and (5) covalently closed supercoiled monomer forms of pQSR50. Positions of molecular size markers (*Hind*III-digested λ phage DNA) are indicated.

and pQSR50 had significantly different transforming activities, both from RSF1010 and R1162 as well as from each other (Table 2).

Although the plasmids R1162 and RSF1010 yielded the highest transformation frequencies, spontaneous

Fig. 2. Transformation frequency as a function of the amount of plasmid DNA per filter using an untreated plasmid preparation (∇), a unique site restriction enzyme digested plasmid preparation ($\bullet$) or a plasmid preparation enriched for oligomers ($\blacktriangledown$).

Fig. 3. Transformation of *Vibrio* strain WJT-1C as a function of length of time of exposure to pQSR50 DNA.

Fig. 4. Development of competence in *Vibrio* strain WJT-1C. $\bullet$, Transformation frequency; $\circ$, growth of culture (OD₆₀₀).

resistance to streptomycin occurred in WJT-1C at a frequency of 3.1×10^{-7} . Therefore, we chose to use pQSR50 as transforming DNA in all following investigations since the rate of spontaneous mutation to WJT-1C to both streptomycin and kanamycin was below 10^{-9} (data not shown).

Concentration and form of transforming plasmid DNA

The amount of plasmid DNA required for transformation of WJT-1C and the relationship between the transforming activity of an untreated plasmid preparation to those which had been enriched for either linear or oligomeric forms was investigated. Fig. 1 shows an autoradiograph of a Southern blot (Sambrook *et al.*, 1989) of the plasmid preparations used. Filters were hybridized to the ^{35}S -labelled probe pNPTII, which hybridizes to the neomycin phosphotransferase gene of Tn5 contained on pQSR50 (Frischer *et al.*, 1990). None of the preparations were composed solely of one topological form. The untreated plasmid preparation (lane A) was composed primarily of relaxed (band 2) and supercoiled (band 5) forms. These two forms were distinguished from each other by a topoisomerase assay (data not shown) performed as recommended by the manufacturer (Promega Corporation). Preparation B was composed primarily of linearized pQSR50 (band 4) but also contained a trace amount of what appeared to be linearized dimeric molecules, based on its molecular size (band 1). Preparation C was composed primarily of covalently closed oligomeric molecules (band 3) but also contained linear monomer and dimer molecules. The transformation activity of each of these plasmid preparations was assayed. Transformation was observed in all preparations and increased exponentially with concentration until saturation at $1.0 \mu\text{g}$ DNA per filter (Fig. 2). Transformation was observed at the lowest concentrations of DNA tested (100 ng per filter or approx. 0.1 fg per cell), even in the digested preparation. Below saturating concentrations, preparations enriched in oligomeric molecules exhibited higher transforming activity than did the untreated preparation. At all concentrations tested the plasmid preparation enriched for linear molecules (preparation B) had the lowest transforming activity.

Time required for transformation

Fig. 3 shows the transformation of *Vibrio* strain WJT-1C exposed to pQSR50 multimers as a function of time. Transformants were detected after 1 h of exposure (9.2×10^{-7} transformants per recipient), but were not detected when the exposure period was limited to 30 min. The transformation frequency increased exponentially from 1 h to 4 h, at which point the transformation frequency was 8.3×10^{-5} transformants per recipient. The transformation frequency was only slightly increased by continuing exposure overnight (22 h; 1.4×10^{-4} transformants per recipient). Because these incubations were terminated by DNAase I treatment these results indicate that at least the binding and uptake of transforming DNA resulting in a DNAase I protected

Fig. 5. Maintenance of competence in *Vibrio* strain WJT-1C in (a) artificial seawater with nutrients (5 g peptone and 1 g yeast extract l^{-1}) and in (b) artificial seawater without nutrients. ●—●, Transformation frequency; ■—■, transformants ml^{-1} ; ▽—▽, total c.f.u.; □—□, DAPI direct counts. Bars indicate SD for cells ml^{-1} determinations (not shown where smaller than symbol).

condition occurs within this time period, similar to the transformation process in other organisms (Contente & Dubnau, 1978; Albano *et al.*, 1987; Seifert *et al.*, 1988).

Development of competence

Fig. 4 shows the onset of competence in *Vibrio* strain WJT-1C. Transformation could be detected at the earliest growth phase assayed (early exponential) and it increased exponentially from 3.5×10^{-7} transformants per recipient in the early-exponential phase to 2.8×10^{-5} transformants per recipient in the late-exponential phase. Maximal transformation frequencies occurred during the stationary phase (1.8×10^{-5} transformants per recipient).

Maintenance of the competent state

Fig. 5 shows the maintenance of competence in cultures of *Vibrio* strain WJT-1C which were kept for up to 10 d in spent rich medium (Fig. 5a) or in starvation medium (Fig. 5b). Total viable counts, DAPI direct counts, transformants ml^{-1} , and transformation frequency were

Fig. 6. Effect of temperature, salinity and nutrient concentration on the competence of *Vibrio* strain WJT-1C. (a) Effect of temperature. ○—○, Transformation frequency determined after 4 h exposure to plasmid DNA; ●—●, after 20 h exposure to plasmid DNA. (b) Effect of salinity. (c) Effect of nutrient concentration. Nutrient concentration expressed as a percentage of the standard peptone (P) and yeast extract (Y) concentration (100% PY = 5 g P, 1 g Y l^{-1}). Bars in (c) indicate SD (not shown where smaller than symbol).

measured over the incubation period. Competence was maintained at high levels in both spent and nutrient-free media for the 10 d duration of the experiments. In spent nutrient medium, transformation frequency decreased from 5.5×10^{-5} to 6.6×10^{-6} transformants per recipient over the 10 d period, while in nutrient-free medium the transformation frequency decreased from 3.59×10^{-5} to 2.1×10^{-6} transformants per recipient over the same time period. In both cultures total viable counts, direct counts, and the number of transformants ml^{-1} remained relatively unchanged during the 10 d incubations. These results indicate that competence is maintained at relatively high levels despite a lack of growth.

Effect of temperature

Fig. 6(a) shows the effect of temperature on competence of *Vibrio* strain WJT-1C after 4 or 20 h exposure to plasmid DNA. WJT-1C can grow at temperatures up to

39 °C, with fastest growth rates around 37 °C (data not shown). From 15 to 33 °C, temperature had little effect on the maximum transformation frequency when this was measured after 20 h of exposure, but transformation frequencies measured after 4 h increased with temperature in this range. After 4 h of exposure, transformants could not be detected at either 4 or 15 °C. At 37 °C the maximal transformation frequency was significantly lower ($1.5 \pm 0.01 \times 10^{-6}$ transformants per recipient) compared to maximal transformation frequencies at 29 °C ($7.5 \pm 4.7 \times 10^{-5}$ transformants per recipient). This was true whether exposure to DNA was for 4 h or for 20 h. At 37 °C, competence development was equivalent to that at 29 °C, but transformation frequencies were approximately 100-fold lower during all growth phases (data not shown).

Effect of salinity

Fig. 6(b) shows the effect of salinities ranging from 6‰ to 63‰ on the competence of WJT-1C. Transformants were detected at all salinities, with lowest transformation frequencies at 6‰ (3.9×10^{-7} transformants per recipient) and highest at 22‰ (8.4×10^{-5} transformants per recipient). From 22 to 51‰ there was a small decrease in transformation efficiency to 3.2×10^{-5} , and there was a further decrease to 4.85×10^{-6} transformants per recipient at 63‰.

Effect of nutrients

Nutrient concentrations ranging from 1% to 200% of standard PY concentration had no significant effect on the frequency of transformation (Fig. 6c), although higher nutrient levels stimulated the growth of both transformants and non-transformants (data not shown).

Discussion

The induction and maintenance of a competent state are important both for the comparison of the HFT model to other transformation models and to determine favourable conditions for natural transformation in the environment. The development of competence in the HFT *Vibrio* began during early-exponential phase, and once attained was maintained for at least 10 d. Furthermore, competence was unaffected by starvation conditions and could only be reduced by allowing the cells to return to exponential growth. This pattern is in contrast to competence development patterns in other transformation models. Competence in most other organisms appears to be a tightly regulated process, occurring only during late-exponential or stationary phase for a short period of time, after which competence declines dramatically (Smith *et al.*, 1981; Stewart &

Carlson, 1986; Stewart, 1989). Exceptions include *Streptococcus pneumoniae*, in which a low level of competence develops in mid-exponential-phase cultures but is not maintained after maximal competence is achieved in late-exponential phase (Pakula & Walczak, 1963; Tomaz & Hotchkiss, 1964), and *Neisseria*, in which competence is constitutive in piliated strains (Sparling, 1966). Competence in *Acinetobacter calcoaceticus* strains derived from strain BD413 (Juni, 1972) has recently been shown to be constitutively expressed, but competence in this strain declined rapidly during 30 h incubation in sterile river water (H. G. Williams, M. J. Day, J. C. Fry & G. J. Stewart, unpublished results). This work was in contrast with earlier work with *A. calcoaceticus* strains BD413 and NCIB 8250, in which competence was found only during the mid-exponential growth phase (Ahlquist *et al.*, 1980; Cruze *et al.*, 1979). Thus, the HFT *Vibrio* WJT-1C seems to be unique with respect to competence development and maintenance.

Mutants of *Haemophilus influenzae* and *S. pneumoniae* have been isolated which exhibit enhanced competence (Redfield, 1991; Lacks & Greenberg, 1973). However, the similarity of these mutations to the HFT mutation is unclear since HFT strains seem not only to exhibit enhanced competence during early-exponential phase but also during stationary phase (Frischer *et al.*, 1990). Additional investigations are required to determine the nature of these mutations in *H. influenzae*, *S. pneumoniae*, and in *Vibrio*. It may be that these marine *Vibrio* HFT strains do not possess transformation systems that are under the control of a specific regulon. Rather, a mutation in any number of cellular functions (e.g. membrane permeability, macromolecular transport, nuclease activities, or any DNA-processing function) may give rise to the competent phenotype. If this is the case then the establishment of heterologous DNA by transformation may be more common than is indicated by the rarity of competent organisms, especially if plasmids which provide selective advantages are involved.

The effects of temperature, salinity, and nutrient concentration on plasmid transformation frequency in *Vibrio* strain WJT-1C were minimal. Significant reduction from the maximal transformation frequency was observed only in conditions that rarely occur in estuarine environments. This observation suggests that estuarine environments provide conditions which facilitate plasmid transformation. Similar conclusions have been reached concerning the process of conjugation in environmental isolates (Rochelle *et al.*, 1989; Sambri & Lovett, 1989). The stability of transformation frequency over a wide temperature range indicates that the transformation process is independent of the growth rate, substantiating the observation that transformation occurs normally

during periods of unbalanced or stationary growth. Interestingly, competence was significantly reduced at 37 °C. Cells which were grown and transformed in a variety of different salinities (12–50‰) exhibited only small differences in transformation frequencies, demonstrating that transformation of this organism could occur equally efficiently under the wide range of salinities that might normally be encountered by this organism. As with temperature and salinity, the concentration of nutrients in the growth media had little effect on the transformation frequency of WJT-1C, although the nutrient level greatly affected the culture yields. The pattern of competence development and the flexibility in culture conditions which support transformation suggests that HFT marine *Vibrio* strains could readily acquire plasmids via natural transformation under a variety of circumstances, especially during starvation or even during the seemingly dormant physiological state termed the 'starvation state' (Morita, 1985).

Plasmid oligomers were used in these studies as transforming DNA because they contain self-homology, believed to be required for homologous recombination (Stewart, 1989; Saunders & Saunders, 1988). Enrichment for oligomers in the plasmid preparations increased the transforming activity, suggesting that, as in other transformation systems, homologous recombination facilitates plasmid establishment in HFT marine *Vibrio* strains. Regardless of the plasmid preparation used, the transformation frequency was dependent on the amount of DNA available and the process could be saturated, possibly indicating the existence of DNA-binding sites. Alternatively, saturation may be an artifact of the assay, which probably limits transformation to organisms on the surface of the cell spot.

A critical question concerning the potential for natural transformation as a route of gene exchange in the marine environment is whether there are sufficient concentrations of dissolved DNA in marine environments to act as transforming DNA. The results from this study demonstrated transformation even at the lowest DNA concentrations used (100 ng per filter; 10^9 cells per filter). This concentration is within the range of DNA concentrations reported for marine environments (DeFlaun *et al.*, 1987; Ogram *et al.*, 1987). However, it is difficult to extrapolate these results to the natural environment since there are no estimates of the fraction of dissolved DNA that represents DNA with transforming potential and only a few estimates of the fraction of marine bacteria that are competent for natural transformation (Stewart & Cyr 1987; M. E. Frischer, unpublished results). Thus, the implications are that sufficient dissolved DNA concentrations for transformation exist in estuarine waters, but further characterization of dissolved DNA pools and competence in

marine micro-organisms with marine plasmids will be necessary before we can better extrapolate our results to the marine environment.

Another important consideration concerning the potential for transformation in the environment is the comparison between the rate of degradation of DNA and the rate at which it can be taken up for transformation. The results presented in this work indicate that transformation, or at least the sequestering of transforming DNA into a DNAase-protected form, is rapid, occurring on a time scale of minutes to hours, while rates of DNA turnover in marine surface waters and sediments have been reported to be in the order of hours to days (DeFlaun & Paul, 1989; Paul *et al.*, 1987; Bazalyan & Ayzatullin, 1979; Novitsky, 1986; Maeda & Taga, 1974). Therefore it may be speculated that the turnover rate of DNA is not the primary limiting factor for transformation in marine environments and that depending on other parameters (e.g. cell density, DNA availability, presence of inhibitory agents) natural transformation may occur readily.

These studies indicate that the HFT marine *Vibrio* strain WJT-1C possesses transformation characteristics not observed in other transformation systems. The transformation system of this marine *Vibrio* enabled transformation to occur under highly variable conditions normally associated with natural environments. The ability of this strain to maintain a competent state for long periods and the speed at which it can sequester DNA support our earlier observations from microcosm studies that natural transformation could allow for the transfer of small plasmids in the marine environment.

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